Phthalate Screening in Food Packaging by Ambient Ionization High Resolution MS/MS Mass Spectrometry

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Overview

Purpose:

Demonstrating the analysis of phthalic acid diesters on a high resolution accurate mass Orbitrap™ analyzer based system coupled to a "Direct Analysis in Real Time" (DART™) ionization source. Due to the presence of a quadrupole mass filter and a collision cell, the system is capable of selective precursor isolation for higher energy collision induced dissociation (HCD) in order to distinguish different isomers and to confirm possible positive hits.

Methods:

A "Direct Analysis in Real Time" (DART™) ionization device was coupled to an Orbitrap™ based mass spectrometer in order to directly analyze different samples without the need for sample preparation and to avoid phthalate contamination during preparation steps. The background spectra were carefully monitored for ambient phthalate background levels during sample analysis and for instrument inlet contamination. Results of Full MS analysis and HCD fragmentation of standards were compared with spectra obtained from commercial food packaging and food contact materials. Specific MS2 fragments were used to confirm the presence of banned substances.

Results:

Phthalic acid diester standards could be distinguished by their accurate mass in full scan analysis and their specific fingerprint after performing Full Scan MS2 analysis. Screening of the food packaging materials was done in less than 2 minutes per sample.

Introduction

Phthalic acid diesters (PAEs), also know as phthalates, are widely used in industry as plasticizers in everyday products like toys, flooring, personal care products and food packages. These compounds can be present up to a high ratio in some materials. As substances classified as semi-volatile organic compounds (SVOC), they are evaporating into the environment over a long time.

Some of the PAEs have been classified being hazardous, affecting mainly the reproductive system and might as well increase the risk of cancer [1,2]. The use of these compounds is officially under regulation but still, these phthalates might be present and used during production of goods.

Former studies using high resolution accurate mass (HRAM) mass spectrometry were performed on a benchtop orbitrap system in full scan mode [3]. In the presented study, advantage was taken from the possibility using precursor selection for MS2. Obtaining a full scan MS2 spectrum, the occurring fragmentation pattern could be used as a fingerprint for the characterization of the different compounds, especially of the different isomers for nominal mass to charge ratios 279 and 391.

Due to their ubiquitousness in indoor environments, the cross contamination during analysis has to be kept as low as possible. Main source of contamination are glass ware and organic solvents. Therefore, a direct examination without the need of sample extraction and chromatography is of great advantage for these kind of analysis. Still, background coming from carpets etc. has to be critical monitored and subtracted from the sample derived signals.

Methods

Mass Spectrometry:

All data was acquired using a Q Exactive™ mass spectrometer (Thermo Scientific, Bremen, Germany; Figure 1) coupled to a direct analysis in real time DART SVPA ion source (IonSense Inc., Saugus, MA, USA). The DART source was operated at 200ºC using helium as carrier gas.
Source settings for the mass spectrometer were 200ºC for the capillary transfer tube and an S-Lens Level of 50 arb.u.

The mass spectrometer was operated in full scan mode (positive ionization mode, mass range m/z 100-1000, AGC target 1e6 charges, R=140k), SIM mode (positive ionization mode, isolation width at full width half maximum 2u, AGC target 1e5 charges, R=140k) as well as MS2 mode (positive ionization mode, isolation width at full width half maximum 2u, AGC target 2e5 charges, R=140k).
For HCD fragmentation, the normalized collision energy (NCE) was set to 10 arb.u.
Targets were chosen for inclusion list with regards to Table 1.
All scan events were scheduled in one method setup resulting in a total method duration of 1.2 minutes (Figure 2). After starting the acquisition, no sample was introduced up to 0.3 minutes in order to get the background level. Samples were introduced at 0.3 minutes starting with the full scan analysis.

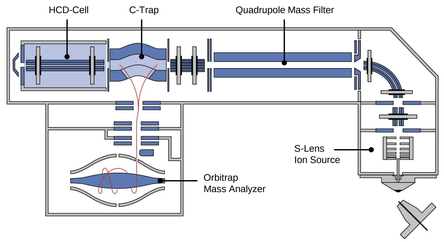

FIGURE 1. Schematics of Q Exactive™ instrument.

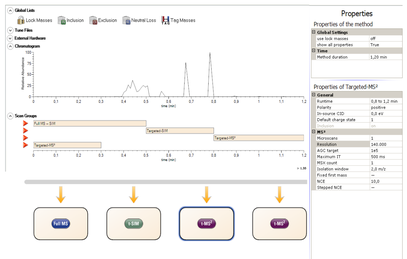

FIGURE 2. Method setup for the phthalate screening method. The method is including full scan analysis as well as targeted SIM and targeted MS2 for the chosen compounds of interest (Table 1). The first 0.3 minutes were scheduled in order to get the background level information without introducing the samples.

Compound	Elemental composition	Precursor [M+H]+	Selection of characteristic HCD fragments m/z	Regulation
Di-n-butyl phthalate (DBP)	$C_{16}H_{22}O_4$	279.1591	167.0339; 205.0859; 223.0965	CA Prop 65
Diisobutyl phthalate (DIBP)	$C_{16}H_{22}O_4$	279.1591	167.0339; 205.0859	EU
Benzyl butyl phthalate (BBP)	$C_{19}H_{20}O_4$	313.1434	91.0542; 205.0859	CA Prop 65
Bis(2-ethylhexyl)phthalate (DEHP)	$C_{24}H_{38}O_4$	391.2843	167.0339; 279.1591	CA Prop 65
Di-n-octyl phthalate (DnOP)	$C_{24}H_{38}O_4$	391.2843	167.0339; 261.1485	CA Prop 65
Dioctyltere phthalate (DOTP)	$C_{24}H_{38}O_4$	391.2843	167.0339; 261.1485; 279.1591	-
Diisononyl phthalate (DINP)	$C_{26}H_{42}O_4$	419.3156	127.1481; 275.1642; 293.1747	CA Prop 65
Diisodecyl phthalate (DIDP)	$C_{28}H_{46}O_4$	447.3469	141.1638; 289.1798; 291.1955; 307.1904	CA Prop 65

TABLE 1. List of analyzed phthalate standards. Exact masses of [M+H]+ for precursors as well as for a selection of characteristic HCD fragments are displayed.

Sample preparation

Standards were purchased from Sigma Aldrich (St Louis, MO,USA) and were applied as liquids onto stainless steel mesh (Figure 3). Standards were introduced to the DART source using a motorized sample platform. The commercial packaging samples were introduced to the source by tweezers using an adjustable tweezer base in order to maintain a distinct distance from sample to instrument inlet (Figure 4).

FIGURE 3. DART SVP ionization source attached to a Q Exactive system. The tweezer is showing a lid gasket sample shortly before sampling.

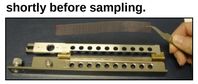

FIGURE 4. Sampling set-up for phthalate standards for DART analysis. Stainless steel mesh screens used as the sampling surface for phthalate standards. Deposited liquid volume was about 3 µL.

Results

Standards and background ions

Phthalates are ubiquitously distributed in lab environments. Phthalate contamination during sample preparation might mimic or disturb the actual results. Using DART ionization, these contaminations can be minimized as a results of no sample preparation required for the sample analysis. Background signal was measured prior to each sample acquisition in order to monitor for ambient phthalates that could be detected with the sample.
The generated HCD fragments (Figure 5-8) were in accordance to the fragments obtained on a linear ion trap (data not shown) and to those described in the literature [4,5]. With regards to product ion ratios, the HCD fragmentation is more harsh whereas the intensities of higher m/z species is lower. M/z 149 is MS2 base peak for all standards except DiDP. With the missing low-mass cut-off while using HCD, these lower m/z product ions could be obtained (Figure 7).

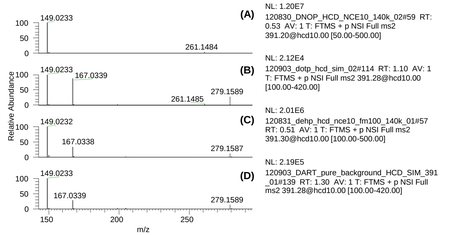

FIGURE 5. HCD spectra of DNOP (A), DOTP (B) and DEHP (C) standards. The lower panel is showing a background spectrum acquired prior to the measurements (D). All sample compounds show a precursor m/z of 391. 2843. Sample introduction was done 1min after starting data acqusition. Spectra are showing the presence of m/z 261 and absence of m/z 167 as specific marker for DNOP. All measured mass-to-charge ratios are within a mass deviation of ≤1.4 ppm compared to the calculated exact masses. Data was acquired with external mass calibration.

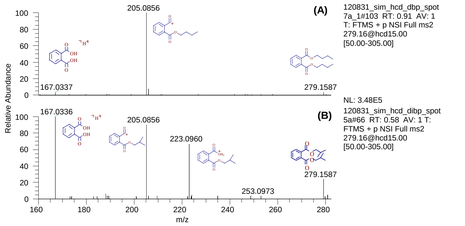

FIGURE 6. HCD spectra of DBP (A) and DIBP (B) standards. Zoom in to mass range 160-250. Proposed HCD fragments are assigned using Mass Frontier™. All measured mass-to-charge ratios are within a mass deviation ≤2.2 ppm compared to the calculated exact masses. Data was acquired with external mass calibration.

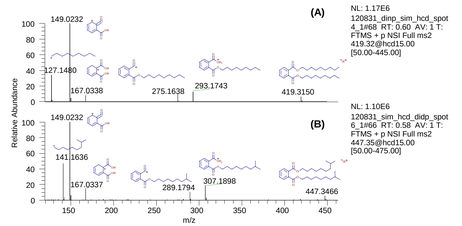

FIGURE 7. HCD spectra of DINP (A) and DIDP (B). Proposed HCD fragments are assigned using Mass Frontier™. All measured mass-to-charge ratios are within a deviation ≤1.9 ppm compared to the calculated exact masses. Data was acquired with external mass calibration.

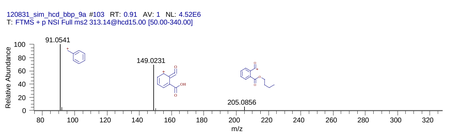

FIGURE 8. HCD spectrum of BBP. Proposed structures are assigned using Mass Frontier™. All mass-to-charge ratios are within a mass deviation ≤1.5 ppm compared to the calculated exact masses. Data was acquired with external mass calibration.

Food packaging material and everyday products

In total, 13 lid gaskets, 9 milk packages, 5 bags and fruit containers were tested. In addition, 7 plastic shoes, 12 wallet samles and 2 sports equipment were tested as well. No positive hit for one of the tested standard compounds could be found in the samples. Full MS data reveals the presence of other compounds used in polymer and plastic industry like acetyl dibutyl citrate (ATBC), diethylhydroxylamine (DEHA), acetylated partial glycerides (AcPG) and erucamide could be identified (data not shown). For future work, standards have to be prepared containing defined ratios of standards for going on with the quan approach.
The presented screening method offers a very fast and convenient setup for getting high resolution accurate mass full scan data as well as getting the whole MS2 fragmentation pattern with the same quality.

Conclusion

- DART™ combined with Orbitrap™ based HRAM LC-MS/MS was shown to be a very fast and convenient way for screening for additives in food packaging and other goods
- Due to ambient phthalate content the background has to be carefully monitored before starting the analysis of each sample to avoid contamination
- For future work, defined solid standards have to be prepared to go on with the quantitative approach

References

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