

Determination of Contaminants in Wastewater with a Benchtop Single Stage Orbitrap Mass Spectrometer

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Overview

Purpose: Detection of contaminants in wastewater

Methods: Detection and quantitation based on full scan MS data and confirmation based on accurate mass/isotopic patterns using a novel benchtop Orbitrap mass spectrometer

Results: Linear range of quantitation covering four orders of magnitude and reliable elemental composition calculation based on accurate mass and isotopic patterns

Introduction

The number of samples in environmental analysis is constantly growing. In addition, the number of analytes to be monitored has increased year by year. So far, mass spectrometry based on triple quadrupole technology is the technique of choice for these analytical tasks because of its high sensitivity and selectivity. The wide variety and large number of compounds to be measured has brought up the demand for higher mass spectrometric productivity but this technique is still limited in speed of analysis and in the number of analytes which can be monitored simultaneously. When the analysis is done in MS full scan mode, the number of analytes is not limited and the search for so far unknown compounds is possible. Also post acquisition re-interrogation becomes possible. The use of a mass spectrometer, capable of fast polarity switching in combination with full scan high resolution providing mass accuracy in both polarity modes can enhance productivity in the analysis of environmental samples. Full sample analysis in one chromatographic run becomes possible, providing high sensitivity and selectivity, comparable to standard MS techniques.

Methods

Sample preparation

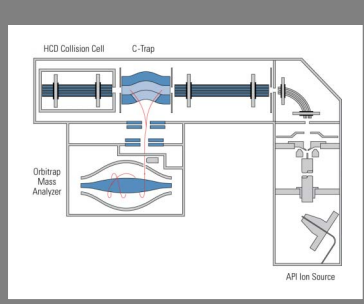
Crude waste water samples from a chemical plant were spiked with a synthetic mix of 136 possible contaminants at different levels and used as this for injection into the chromatographic system.

LC-ESI-MS

All spectra were acquired on a Thermo Scientific Exactive LCMS instrument equipped with an Accela™ U-HPLC system using a 3 μm 150x2.1 mm Hypersil GOLD™ C8 column at a flow rate of 300 μl/min. An 8-minute gradient formed from water and methanol (1 mM ammonium acetate added to both solvents) was applied for best separation of the analytes. Electrospray ionization was performed using a heated electrospray probe. The whole method time added up to 15 minutes (see Figure 2). The Exactive™ single stage Orbitrap mass spectrometer was set to a resolution of 25,000 (FWHM at *m/z* 200) collecting alternate positive and negative mode full scan data.

This resulted in a full cycle (one positive and one negative spectrum) in less than one second ensuring enough data points across the chromatographic peaks. Mass calibration was carried out as external calibration.

FIGURE 1.
Diagram of the Exactive mass spectrometer



Results

The Exactive is a single stage Orbitrap mass spectrometer having no precursor ion selection device by design. All ions produced in the ion source are directly transferred to the Orbitrap mass analyzer. This design ensures that the full range of ions produced in the ion source reaches the curved shape linear ion trap for collection and injection into the mass analyzer (see Figure 1). In the optional HCD collision cell, the collected ions can be fragmented with subsequent detection in the Orbitrap mass analyzer.

FIGURE 2.

Selection of analytes

aniline	2-Mercyphenol	dimethyl adipate
2-methylamin	2,2-bis(4-hydroxyphenyl)-propane	diisooctyl adipate
3-methylamin	2-chlorophenol	dibenzyl adipate
N-methylamin	m-chlorophenol	benzoyloctyl adipate
N,N-dimethylamin	4-chlorophenol	dimethyl phthalate
N,N-diethylamin	4-chloro-3-methylphenol	diethyl phthalate
N-ethyl-3-methylamin	6-chloro-3-methylphenol	monomethyl phthalate
diphenylamin	4-chloro-o-cresol	di-n-butylphthalate
benzylideneamin	2,4-dichlor-o-cresol	dicyclohexyl phthalate
2-amino(diphenyl)amine	4,6-dichlor-o-cresol	Phthalate/benzylbutylester
4-amino(diphenyl)amine	3,5-dinitro-2-methylphenol	butyric acid
3-hydroxy(diphenyl)amine	2,4-dinitro(diol)	isobutyric acid
2,2'-diamino(diphenyl)methane	2,2-dimethyl-1,3-propanediol	valeric acid
2,4'-diamino(diphenyl)methane	C5 diol	isovaleric acid
4,4'-diamino(diphenyl)methane	C7 diol	caproic acid
acridine	bispropene	n-heptanoic acid
tricyclamin	bisformal	n-caproic acid
ethyl piperidine	1,1,1-Tris-(hydroxymethyl)-propane	2-ethylhexanoic acid
cyclohexyl amin	benzyl alcohol	pelargonic acid
dicyclohexyl amin	3,3,5-trimethyl hexanoic acid	succinic acid
epilone-Caprolactam	2-chlorobenzyl alcohol	glutaric acid
phthalimid	4-chlorobenzyl alcohol	adipic acid
N-phenylsuccinamide	1,6-hexane diol	palmitic acid
N-phenylsuccinamide	trans-1,2-Cyclohexane diol	palmitoleic acid
triethyl phosphate	1,3-cyclohexane diol	margaric acid
tributyl phosphat	cyclopentanone	

Optimum conditions for the given task of screening and quantification was a mass resolution of 25,000 FWHM with permanent positive/negative switching in terms of speed of analysis and confidence of the results. Comparison to positive only and negative only full scan experiments showed no difference in sensitivity and mass accuracy. No component-specific tuning is needed for this instrument, so after setting up the chromatographic system all measurement could be carried out without further delay.

The synthetic mix of contaminants spiked to the samples contained some components which were best analyzed in negative mode (i.e., phenols or acids), while other components clearly were only visible in positive mode. To achieve highest sample throughput in routine analysis, all components had to be analyzed in one chromatographic run.

The best chromatographic result was found under non U-HPLC conditions, providing good compound separation in a total run time of only 15 minutes. Together with the mass spectrometric conditions this resulted in a minimum of 15 data points across the chromatographic peak (see Figure 4).

In a sample spiked with an amount of 500 ppb for each component, up to 111 of 136 compounds could be detected. Figure 3 shows extracted ion chromatograms of selected compounds in a sample spiked with 5 ppb of each compound. Still 100 analytes could be identified in this sample. Identification was carried out by generating extracted ion chromatograms with a 5 ppm window around the theoretical mass of the spiked compounds by means of Thermo Scientific ToxID automated software. Quantitation was carried out using Thermo Scientific LQUAN software, and showed reproducible results for all identified compounds.

FIGURE 3.

Detection of selected components at a level of 5 ppb in matrix

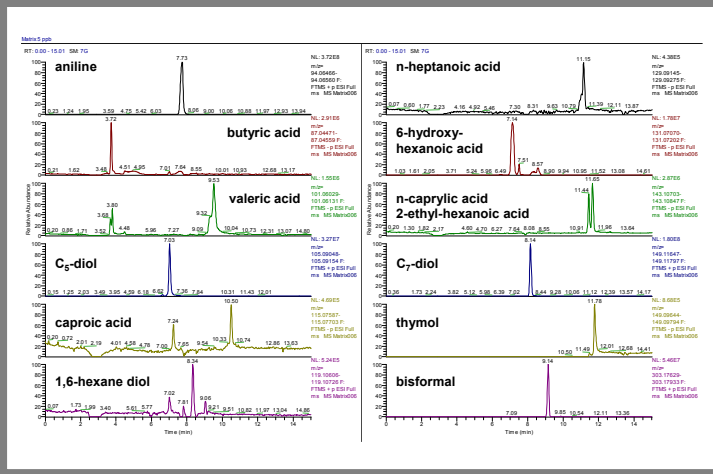
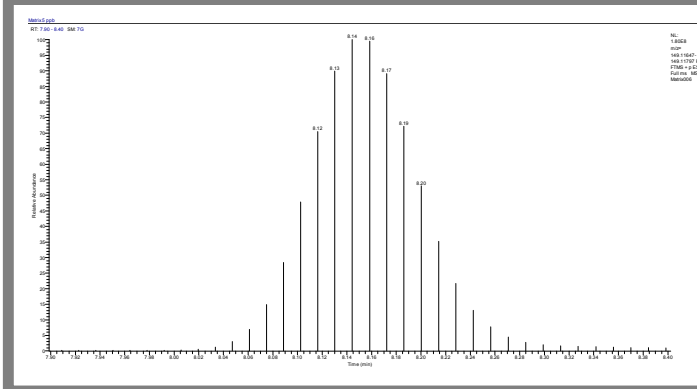


FIGURE 4.

Number of data points across a chromatographic peak with polarity switching (the peak of C₇-diol from Figure 3 is shown)



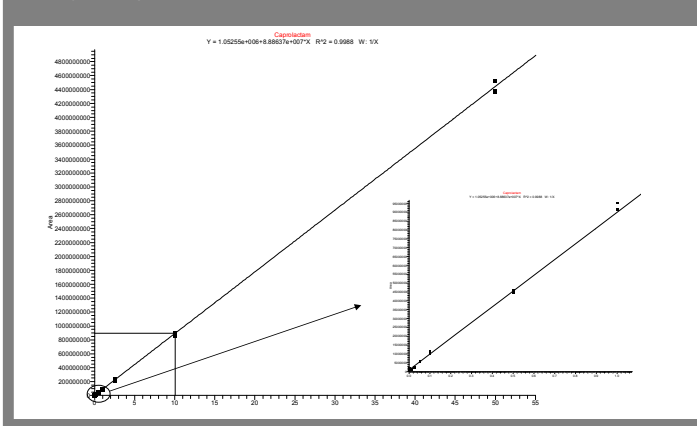
Linearity was tested for all compounds over a range from 5 ppb to 50 ppm. Some compounds showed non linear behavior in the range of 10 to 50 ppm, but this effect changed with differing chromatographic conditions, so this seemed more likely an issue of overloading of the chromatographic system together with matrix interference.

Most of the compounds gave linear response as shown in Figure 5. Even for the critical compounds a range of linearity of more than 3 orders of magnitude could be ensured.

To gain further confidence in the identification beyond accurate mass and retention time of the chromatographic system, the isotopic pattern of the identified masses can be used for sum formula determination.

FIGURE 5.

Linearity and range of quantitation (5 ppb to 50 ppm)



As shown in Figure 6, even a minor component (marked with a blue circle) being two orders of magnitude smaller than the most abundant signal of the scan showed good and precise isotopic information for reliable identification of the signal.

FIGURE 6.

Elemental composition determination with isotopic pattern recognition (n-heptanoic acid taken as an example)



Conclusions

We demonstrated the analysis of a broad range of contaminants in chemical plant wastewater spiked with an artificial mix of 136 contaminants. Operating the mass spectrometer in the alternating polarity switching mode allowed for best analytical setup independent on the compound. Full scan high resolution accurate mass data was generated in both polarity modes. Using a 15-minute HPLC method, detection of up to 111 contaminants in one single run was achieved. The accurate mass data achieved in both polarities allowed automated component identification and subsequent quantification. Using external mass calibration, mass accuracies in the low to sub ppm range were achieved in both polarity modes without spectrum averaging, allowing for confident component identification. All experiments were performed at a resolution of 25,000, which turned out to be the best compromise between scan cycle time for obtaining sufficient chromatographic data points and separation of the analytes from any matrix interference. To demonstrate the quantitative performance, a dilution series covering more than 4 orders of magnitude was measured getting linear response for most of the components. Isotopic pattern recognition could be shown as a helpful tool in enhancing the confidence in component identification as well as a first step in identification of unknown masses.

Acknowledgements

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