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Operating Electrodynamic Ion Funnels in Wide m/z Transmission Mode for Simultaneous Analysis of Labile and Non-labile Analytes

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Introduction

Electrodynamic ion funnels have been widely used as atmospheric pressure to vacuum interfaces in mass spectrometry for efficient ion collection and guiding in high pressure regimes. Such devices are traditionally operated at fixed confining radio frequency (RF) amplitudes. While this operation mode provides great benefit for many applications, it can impose limitations for analysis of compounds over a wide m/z range or different gas-phase stabilities. In these cases, users either have to divide their methods into ones containing targets with similar RF optima or use a combined method with a setting that represents a compromise between different optimal RF levels.

This work presents a dual ion funnel (iFunnel) LC/TQ instrument with capabilities of changing the RF amplitude on an MRM-by-MRM basis for ultimate performance.

Experimental

Standards and Methods

Analytical standards were purchased from Wellington Laboratories (Guelph, ON, Canada) and AccuStandard Inc. (New Haven, CT, USA) and diluted with LC/MS grade MeOH to obtain a mixture containing 31 perfluoroalkyl substances at 10 pg/µL concentration.

Method parameters were automatically optimized by the built-in Optimizer of the MassHunter 12.1 acquisition software and kept constant (except for iFunnel modes) for data collection. First, samples were analyzed using single iFunnel mode methods, selecting one of the three default iFunnel settings (Fragile, Standard, Large Molecule) offered by the 6495D LC/TQ. Based on the average response of 10 replicate injections, the best iFunnel mode was selected for each analyte, and samples were reanalyzed using a new method with MRM-specific iFunnel RF settings for each target compound. Next, the PFAS mixture was introduced to the MS by direct infusion and precursor ion responses were recorded in SIM mode while ramping the high and low pressure iFunnel RF amplitudes. By plotting the relative response of each target analyte as a function of high and low pressure iFunnel RF amplitudes, iFunnel transmission profiles of each compound were obtained (Figure 3).

Instrumentation

Data was collected using an Agilent 1290 Infinity II LC system updated with the Agilent InfinityLab PFC-Free HPLC Conversion Kit. Mass spectrometric detection was carried out using a 6495D LC/TQ MS. The 6495D's improved iFunnel driver enables faster data

Experimental

acquisition and processing, as well as switching between different iFunnel RF settings within the same acquisition method. Therefore, the best possible performance can be achieved for each analyte without the need of compromising on a single, method-level iFunnel RF setting.
The iFunnel transmission profile mapping involved direct infusion of the standard mixture using a Harvard Apparatus (Model 22) syringe pump. LC and MS conditions are summarized in Tables 1 and 2.

Table 1. LC conditions

Parameter	Value
Columns	• Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm • Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 5 mm, 1.8 µm • Agilent InfinityLab PFC Delay Column, 4.6 x 30 mm
Injection Vol.	1 µL
Mobile Phase	A: Water + 5mM NH_4-acetate B: ACN
Flow Rate	0.4 mL/min @ 35°C
Gradient	Time (min) %B 0.0 10 0.5 10 1.5 20 7.0 55 10.0 100 12.0 100 12.1 10
Stop-/Post-time	12.5 min / 3.0 min

Table 2. TQ parameters

Parameter	Value
Ion Source	AJS
Polarity	Negative
Drying Gas Temp. & Flow	160°C @ 18 L/min
Sheath Gas Temp. & Flow	390°C @ 11 L/min
Nebulizer Pressure	24 psi
Capillary/Nozzle Voltage	2400V/0V
iFunnel Modes and RF settings (HP/LP iFunnel, V_{p-p})	Fragile: 50/50 Standard: 100/100 Large Mol: 210/210

The 6495D LC/TQ system features three iFunnel modes: Fragile, Standard, and Large Molecule (see Figure 1 and Table 2). When combined, these modes offer the most comprehensive and optimal iFunnel transmission for a wide range of target analytes, on a compound-by-compound basis, within a single method.

Based on results from the single iFunnel mode methods (summarized in Table 3), small, carboxylate type PFAS exhibited preference towards low confining RF amplitudes in the iFunnel assembly. This preference increased with decreasing precursor mass. Small perfluorinated carboxylates are typical examples of fragile analytes. They readily undergo fragmentation (most commonly via CO_2-loss) due to RF heating when high RF levels are applied to the iFunnel assembly. For certain compounds, the extent of this fragmentation could be close to 100%. With increasing precursor ion m/z, perfluoroalkyl carboxylates exhibit higher stability and slight preference towards medium RF voltage setting (Standard iFunnel mode). For sulfonate type PFAS, the highest average response was observed when high RF voltages were applied on the iFunnel assembly (Large Molecule mode), however, differences were moderate between different iFunnel modes. A method was successfully created where target compounds were analyzed using their respective optimum iFunnel modes, achieving ultimate sensitivity (Figure 2).

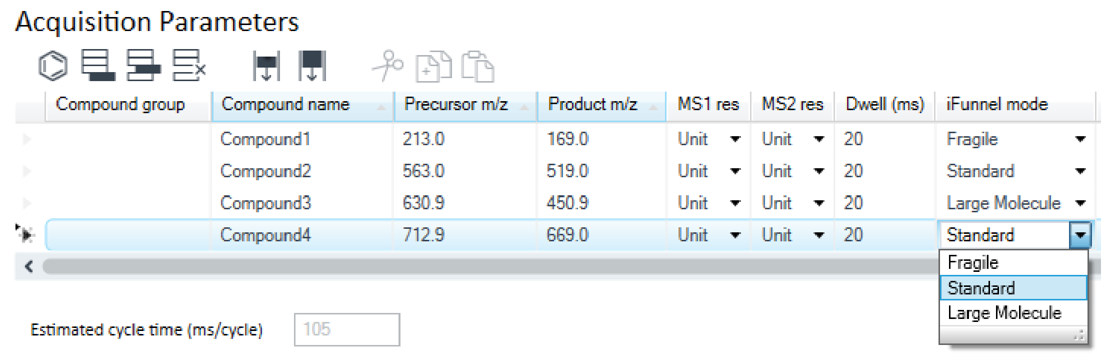

Figure 1. MRM-specific iFunnel settings in the acquisition software for the 6495D LC/TQ

A set of dynamically adjusted inter-MRM delay times was used to account for both ion funnel RF voltage settling time and ion transit time and ensure timely settling of ion signal with changing ion funnel RF amplitude in MRM data acquisition.

Mapping the analytes' iFunnel transmission profiles provided further insight into the observed behaviors (Figure 3). Non-labile analytes have wide, flat maximums on their transmission maps, which explains that they only exhibited slight preference towards any of the three default iFunnel modes. On the contrary, labile ions only have optimum iFunnel transmission under a limited set of applied RF voltages, centered around the Fragile iFunnel mode RF settings. The area of the optimum transmission region, and therefore the exact preference towards Fragile mode is compound dependent.

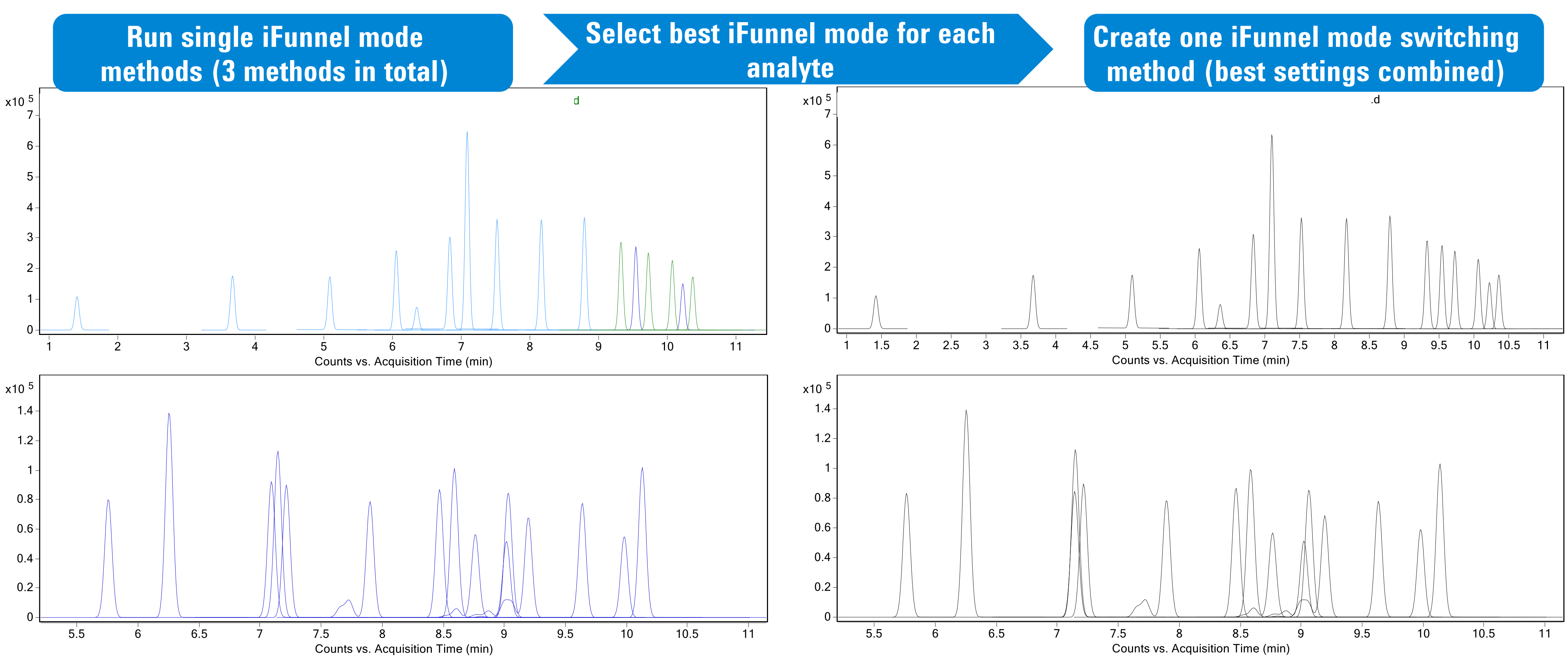

Figure 2. MRM chromatograms of target analytes (perfluoroalkyl carboxylates and ether type PFAS on top, remaining 15 PFAS on bottom). Chromatograms on the left are extracted from single iFunnel mode methods and color-coded to show the best iFunnel setting (light blue – Fragile; green – Standard; dark blue – Large Molecule). Chromatograms on the right (black) are obtained from an iFunnel mode switching method, and show that ultimate sensitivity was achieved simultaneously for each analyte

Results and Discussion

Table 3. Relative responses of target analytes using different default iFunnel modes of the 6495D LC/TQ

Name	Fragile	Standard	Large Mol.	Name	Fragile	Standard	Large Mol.	Name	Fragile	Standard	Large Mol.
Perfluoroalkyl carboxylates (C_3-C_{14})				Perfluoroalkyl sulfonates (C_4-C_{10})				Fluorotelomer sulfonates			
PFPrA	100.0%	77.4%	0.1%	PFBS	94.7%	97.4%	100.0%	4:2 FTSA	94.0%	97.3%	100.0%
PFBA	100.0%	82.4%	0.1%	PFPeS	93.4%	96.4%	100.0%	6:2 FTSA	91.9%	94.2%	100.0%
PFPeA	100.0%	90.3%	0.8%	PFHxS	92.7%	95.0%	100.0%	8:2 FTSA	90.4%	92.1%	100.0%
PFHxA	100.0%	94.7%	4.3%	PFHpS	92.6%	95.6%	100.0%				
PFHpA	100.0%	96.7%	18.3%	PFOS	93.1%	96.9%	100.0%	Perfluoroalkane sulfonamides			
PFOA	100.0%	98.0%	42.6%	PFNS	94.4%	96.6%	100.0%	FBSA	94.6%	98.2%	100.0%
PFNA	100.0%	98.6%	67.0%	PFDS	95.7%	98.7%	100.0%	FHxSA	92.6%	97.0%	100.0%
PFDA	100.0%	98.8%	81.9%	Ether type PFAS				PFOSA	93.1%	98.1%	100.0%
PFUnA	96.1%	100.0%	88.9%	HFPO-DA	100.0%	83.1%	1.0%				
PFDoA	94.1%	100.0%	92.1%	DONA	100.0%	99.7%	48.4%	Perfluorooctane sulfonamidoacetic acids			
PFTrDA	92.7%	100.0%	93.8%	9Cl-PF3ONS	92.3%	96.8%	100.0%	*N*-MeFOSAA	90.9%	94.5%	100.0%
PFTDA	89.2%	100.0%	92.6%	11Cl-PF3OUdS	89.4%	96.3%	100.0%	*N*-EtFOSAA	90.5%	94.6%	100.0%

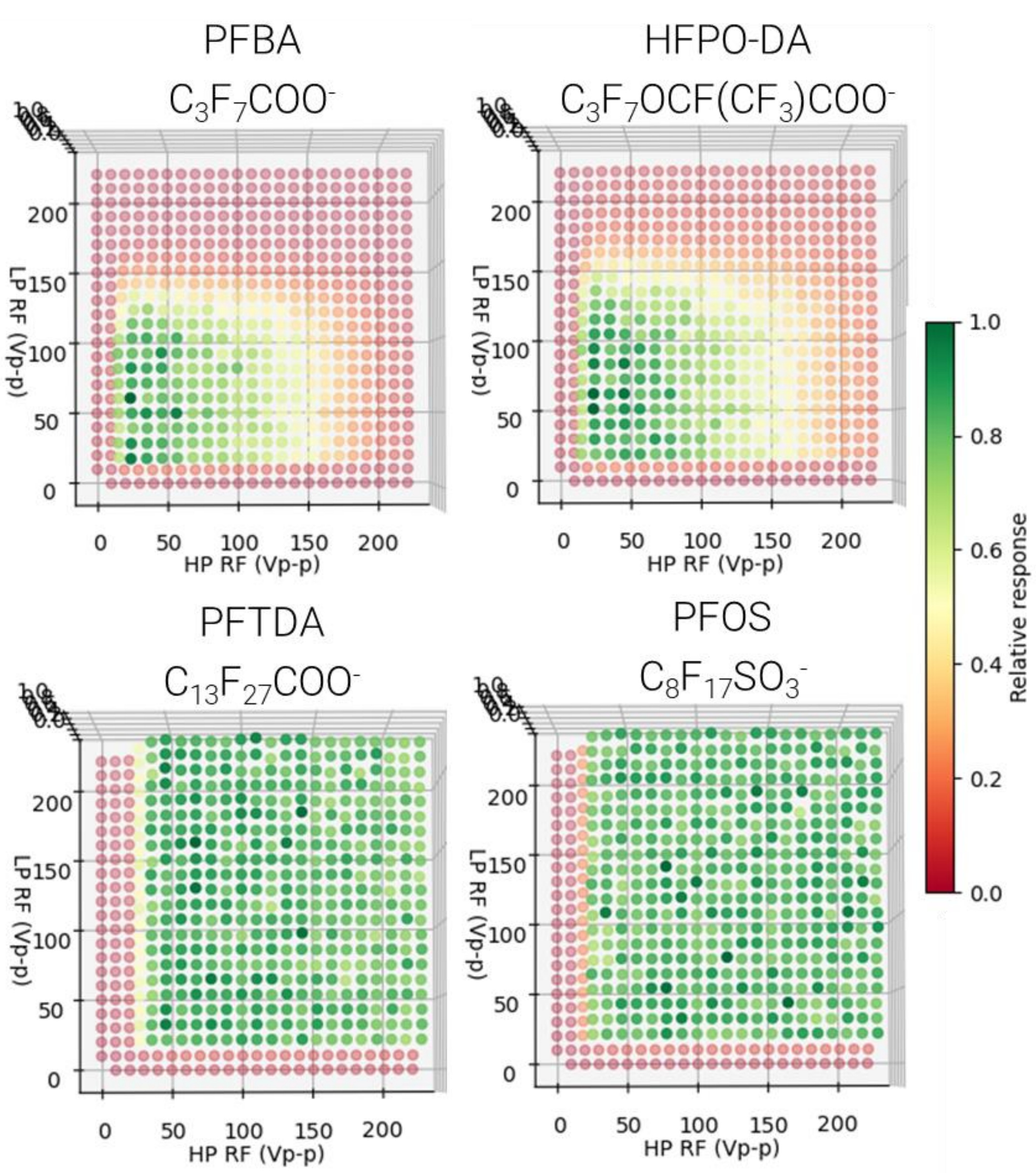

Figure 3. iFunnel transmission maps of two labile (top) and two non-labile (bottom) analytes

Conclusions

Operating a dual-funnel ion guide in RF amplitude-switching mode allows for optimum transmission of targets across a wide m/z range and simultaneous analysis of labile and non-labile analytes for ultimate sensitivity. Three carefully selected iFunnel modes can provide optimum settings for the vast majority of possible analytes. Selecting the optimum iFunnel mode for each analyte can be performed empirically using the approach outlined, and then applied easily in the same method on an MRM-by-MRM basis.

A general recommendation for the three default iFunnel modes available on the 6495D LC/TQ is as follows:

Fragile: Optimized for labile analytes that easily fragment inside the ion funnel or analytes with low m/z precursor ions (approx. m/z < 100)

Standard: Provides optimum transmission for most non-labile analytes with precursor ions in the range of approximately m/z 100-2000

Large Molecule: Optimized for large precursor ions that may need higher RF amplitude to be successfully transferred through the iFunnel (e.g., multiply charged ions of large biomolecules with m/z above approx. 2000)

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Trusted Answers