Application Note: 10262

Analysis of PCBs in Food and Biological Samples Using GC Triple Quadrupole GC-MS/MS

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Key Words

- TSQ Quantum GC
- dl-PCBs
- Food Safety
- Isotope Dilution
- PCBs
- SRM
- WHO-PCBs

Introduction

Polychlorinated biphenyls (PCBs) are a class of extremely persistent industrial chemicals manufactured for use in electrical transformers, capacitors, inks, paints, pesticides, dust control or insulating fluids. Estimates have put the total global production of PCBs on the order of 1.5 million tons. Between 1930 and 1977, the United States was the single largest producer with over 600,000 tons produced. The European region follows with nearly 450,000 tons through 1984.[1,2]

PCBs include 209 distinct chemical forms (congeners), each having different health effects. Although production of PCBs was banned in the United States in 1977, PCB products are still in use. Because of their persistence in the environment, they have been transported around the globe via wind and air currents. PCBs contaminate the bodies of every animal and human being on earth.

The international Stockholm Convention on Persistent Organic Pollutants (POPs) recognizes PCBs among twelve of the world's most dangerous chemicals known to be detrimental to human health and the environment. In spite of the slow but steady decrease of dioxin body burdens, which shows the results of the combined efforts to prevent further distribution, levels of PCBs are expected to stay unaffected globally (Dioxin Conference 2007 Tokyo).[3] Monitoring PCB levels as a part of ongoing programs for the Stockholm Convention will continue for years, with numerous sample requests, particularly for dangerous dioxin-like (dl) PCBs. In particular, coplanar dl-PCBs – non-ortho-substituted PCBs – are the focus of food safety controls due to having a toxicity similar to 2,3,7,8-TCDD. dl-PCBs also contribute significantly to the sample toxic equivalents (TEQ) value.

This application details a fast, reliable and highly selective trace level screening method for the quantitation of PCBs in environmental, food and biological samples, using triple stage quadrupole mass spectrometry with the Thermo Scientific TSQ Quantum GC™. The analytical strategy is analogous to the well-established United States Environmental Protection Agency (USEPA) Method 1668A.[4]

Due to the different analytical response, each chlorination degree is measured against its own isotopically labeled internal standard. This allows for optimal analytical precision and compound similarity. The internal standard compounds are labeled with ^{13}C on the biphenyl backbone, for a total of 12 labels on the biphenyls. The ^{13}C-labeled PCBs are spiked into each sample, which enables accurate identification and correction for the concentration of the native (unlabeled) compounds in the analytical process. This is generally termed "Isotope Dilution Quantitation." A suffix of "L" behind the IUPAC congener number is used to denote the labeled compound; for example, 101L indicates the labeled analogue of the pentachlorobiphenyl congener 101.

Experimental Conditions

Instrument Configuration

Sample analyses were carried out using the Thermo Scientific TSQ Quantum GC GC-MS/MS system, equipped with a Thermo Scientific TRACE GC Ultra™ gas chromatograph. The TRACE GC Ultra was configured with split/splitless injector, and sample introduction was performed using the Thermo Scientific TriPlus™ AS liquid autosampler. The capillary column was a Thermo Scientific TRACE™ TR-Dioxin 5MS column (5% phenyl film) of 30 m length, 0.25 mm inner diameter and 0.10 µm film thickness. Table 1 describes selected instrumental conditions for the GC, autosampler, and mass spectrometer.

TRACE GC Ultra	
Injector:	Split/splitless, 260 °C, 1.2 min splitless
Carrier:	He, constant flow, 0.8 mL/min
Temp. Program:	90 °C, 4 min 15 °C/min, 160 °C 4 °C/min, 225 °C 7 °C/min, 290 °C
Total Run Time:	32.00 min
Transfer Line:	260 °C

TriPlus Autosampler	
Injection Volume:	1.0 µL
Pre-Injection Delay (s):	0.2
Post-Injection Delay (s):	0.2

TSQ Quantum GC	
Source Temp:	240 °C
Ionization:	EI, 40 eV
Emission Current:	100 µA
Q1 Resolution:	0.7 Da
Q3 Resolution:	0.7 Da
Collision Gas:	Argon, 2.0 mTorr
Collision Gas Energy:	22 eV

Table 1: Selected instrument settings for the TSQ Quantum GC, TRACE GC Ultra, and TriPlus Autosampler

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Sample Measurements

USEPA Method 1668 describes a method for the determination of PCB congeners.

> …[Method 1668] was developed by the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology for congener-specific determination of the polychlorinated biphenyl (PCB) congeners designated as toxic by the World Health Organization. Revision A of Method 1668 has been expanded to include congener-specific determination of more than 150 chlorinated biphenyl (CB) congeners. The toxic PCBs and the beginning and ending level-of-chlorination CBs are determined by isotope dilution high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS). The remaining CBs are determined by internal standard HRGC/HRMS. Method 1668A is applicable to aqueous, solid, tissue, and multi-phase matrices.[4]

Commercially available EPA 1668 standards (Wellington, Guelph, ON, Canada) were employed for this application. 68A-CVS is a series of calibration solutions typically used for USEPA Method 1668, Rev. A for HRGC/HRMS. All internal standards (ISTD) were the 12-fold ^{13}C labeled analogues for each PCB chlorination degree. The treatment of samples, internal standards and analytical strategy complied fully with EPA Method 1668A.

TSQ Quantum GC SRM Settings

While USEPA Method 1668 requires the analytes to be "…separated by the GC and detected by a high-resolution (R 10,000) mass spectrometer, [with] two exact *m/z* values …monitored at each level of chlorination (LOC) throughout a pre-determined retention time window", the method described in this application employs a triple quadrupole mass spectrometer equipped with hyperbolic quadrupole rods for increased selectivity, as an alternative approach to HRMS.[4] According to the EU Commission Directive 96/23/EC concerning the performance ranking of analytical methods, the number of identification points of GC-MS/MS methods can be similar or even superior to HRMS, especially for MS/MS techniques using independent product ion transitions (Table 2).[5]

Technique	Number of Ions	Identification Points
GC-MS (EI or CI)	n	n
GC-MS (EI and CI)	2 (EI) + 2 (CI)	4
GC-MS/MS	1 precursor and 2 product ions	4
GC-MS/MS*	2 precursor ions, each with 1 product ion	5
HRMS	n	2n

Table 2: Examples of the number of identification points earned for analytical GC/MS techniques, (n = integer).[3]

** denotes method described here.*

According to the EU Commission Directive 96/23/EC, suitable confirmatory methods for organic residues or contaminants are required to be either full scan techniques or methods that use "…at least 4 identification points (PCBs, dioxins, furans) for techniques that do not record the full mass spectra", which are the common target compound multiple ion detection (MID) methods.[5] By using MS/MS transitions from two PCB precursor ions and detecting individual product ions for each chlorination degree, the measurement scheme in this application follows the EU Commission Directive 96/23/EC and provides five identification points for each PCB. The monitored ion transitions are based on the molecular precursor ions ($^{12}C_{12}H_{10-x}{}^{35}Cl_x$) relative to the mono ^{37}Cl isotopes thereof ($^{12}C_{12}\ H_{10-x}\ {}^{35}Cl_{x-1}{}^{37}Cl$) to form the product ions with a loss of 2 chlorine during the collision induced dissociation (CID) fragmentation process (Table 3). The internal standards follow the same scheme; however, they show a shift of 12 Da due to the 12-fold ^{13}C-labeling.

PCB	Precursor 1 *m/z*	Precursor 2 *m/z*	Product 1 *m/z*	Product 2 *m/z*
MoCB	188.04	190.04	153.04	153.04
MoCB ISTD	200.08	202.08	165.10	165.10
DiCB	222.00	224.00	152.06	152.06
DiCB ISTD	234.04	236.04	164.10	164.10
TrCB	255.96	257.96	186.02	186.02
TrCB ISTD	268.00	270.00	198.02	198.02
TeCB	289.92	291.92	219.98	219.98
TeCB ISTD	301.96	303.96	232.02	232.02
PeCB	323.90	325.90	253.95	255.95
PeCB ISTD	335.92	337.92	265.99	267.99
HxCB	357.80	359.80	287.90	289.95
HxCB ISTD	369.90	371.90	299.51	301.95
HpCB	391.80	393.80	321.90	323.90
HpCB ISTD	403.80	405.80	333.90	335.90
OcCB	427.80	429.80	357.80	357.80
OcCB ISTD	439.80	441.80	369.90	369.90
NoCB	461.70	463.70	391.80	393.80
NoCB ISTD	473.80	475.80	403.80	405.80
DeCB	495.70	497.70	425.80	427.80
DeCB ISTD	507.70	509.70	437.80	439.80

Table 3: SRM data acquisition scheme for PCBs using one precursor ion with the MID detection of two product ions each. The PCB nomenclature is from EPA Method 1668 and reflects level of chlorination.

When choosing precursor ions it should be noted that only the molecular ion M^+, e.g. *m/z* 357.80 $C_{12}H_4{}^{35}Cl_6$ of the monoisotopic HxCB, gives rise to a unique product ion. The next ion of the isotope cluster, *m/z* 359.80, carries one ^{37}Cl which statistically leads to two product ions, one of which gets the ^{37}Cl substitution. This isotope effect leads to lower product ion intensities as the chlorination degree increases.

The analysis sequence in selected reaction monitoring (SRM) mode uses six (6) retention time windows with overlapping masses for all 10 levels of chlorination (LOC). Except for Segment 1, two chlorination degrees were always monitored in parallel. This is due to the staggered elution order of the individual PCB congeners with adjacent chlorine substitution. The high number of masses taken into each SRM analysis segment demonstrates the speed and capacity of the TSQ Quantum GC for parallel multi-component detection. Tables 4 and 5 detail the SRM segments and settings.

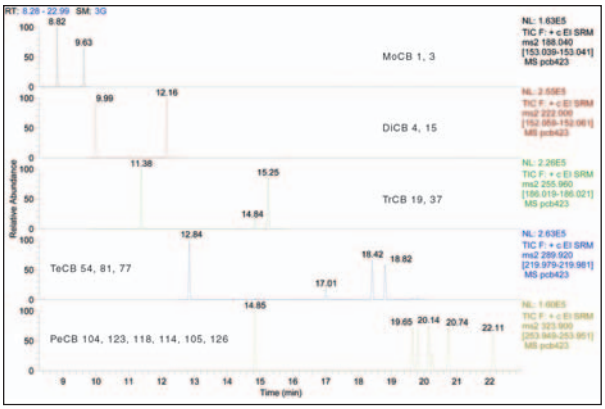

Figure 1: Extracted ion chromatogram showing the congeners with chlorination degrees of mono to penta PCB of a PCB standard (1 pg on-column)

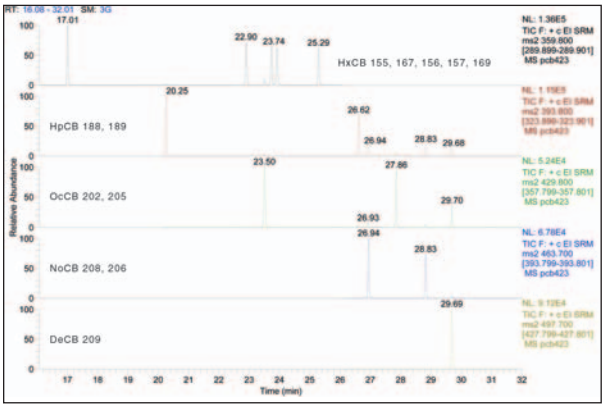

Figure 2: Extracted ion chromatogram showing the congeners with chlorination degrees of hexa to deca PCB of a PCB standard (1 pg on-column)

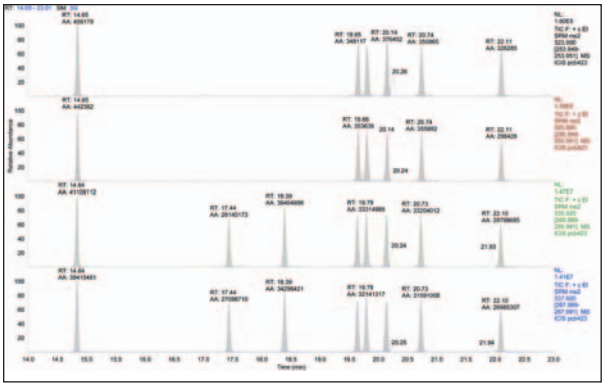

Figure 3: Pentachloro-PCB congeners in standard using two independent SRM transitions each for native (top) and labeled (bottom) PCB congeners 104, 123, 118, 114, 105, 126. The ISTD traces also show the components 101L, 111L.

Results and Discussion

Method Development

All PCB congeners at each chlorination degree were detected using two independent SRM transitions. Each transition used a different precursor ion from the chlorine isotope cluster of the molecular ion region. Data acquisition was performed using the detailed SRM settings described in Tables 4 and 5. MS/MS results for the TSQ Quantum GC are shown in Figures 1 and 2. Figure 1 illustrates chlorination degrees from mono- to pentachloro-biphenyls, while Figure 2 displays the hexa- to decachloro-biphenyl chlorination range. These results were generated using the SRM transitions as described in Table 3. The mass chromatograms in Figures 1 and 2 use the most intense precursor ion for each compound to show the sequence of chlorination degrees. All congeners can be detected at a high response for each SRM transition. The observed decrease in intensity is due to the statistical decrease of the individual isomer concentration as a part of the molecular PCB cluster when injected at 1 pg on-column.

Figure 3 compares the two independent SRM transitions for one chlorination degree. The upper mass chromatograms represent precursors *m/z* 323.90 and *m/z* 325.90 from the native pentachloro-PCB congeners, while the bottom mass chromatograms show the labeled internal standard (precursors of *m/z* 335.92 and 337.92). This comparison demonstrates the excellent consistency between the SRM traces, which allows for confident confirmations of the PCBs.

These SRM mass chromatograms from the TSQ Quantum GC triple quadrupole MS operated in standard resolution mode (0.7 Da peak width) show very good correlation to data achieved using gas chromatography and high resolution mass spectrometry (GC-HRMS). With two independent transitions based on two different precursor ions, the TSQ Quantum GC method meets the high certainty required by the EU directives, as shown for the pentachloro-PCBs in Figure 3. The high speed of the TSQ Quantum GC analyzer also provides an average of 6 to 8 data points across a chromatographic peak, even while monitoring two chlorination degrees in each SRM window. This allows for reliable peak integration and quantitation.

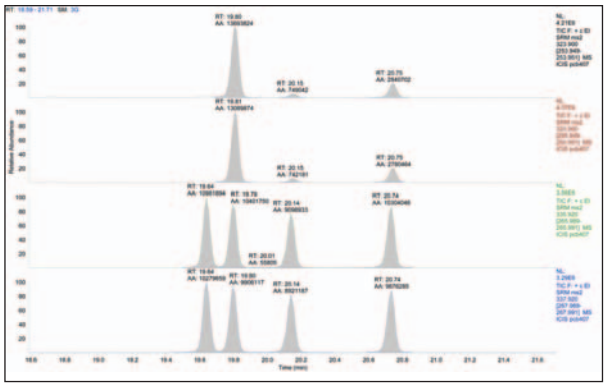

Figure 4: dl-Pentachloro-PCBs in a blood sample

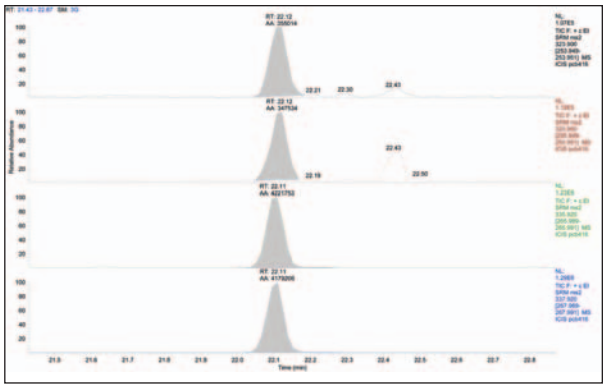

Figure 7: dl-Pentachloro-PCBs in green cabbage

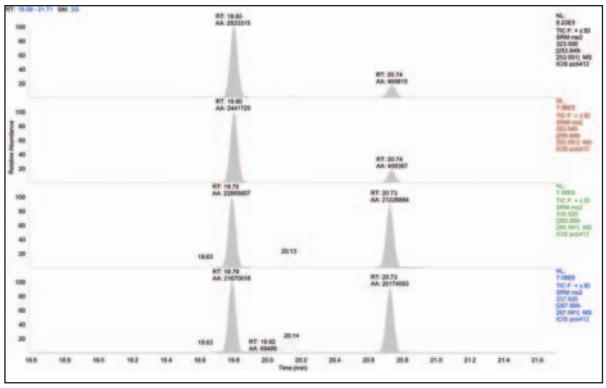

Figure 5: dl-Pentachloro-PCBs in milk

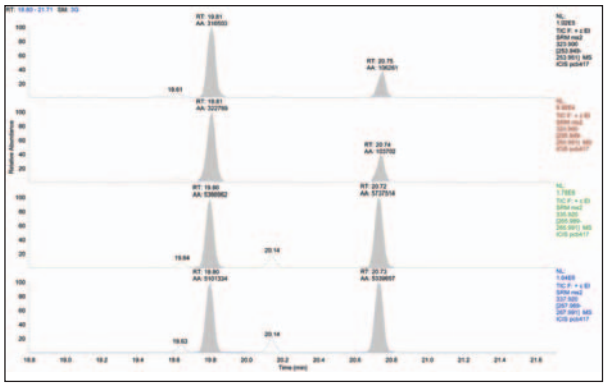

Figure 6: dl-Pentachloro-PCBs in egg yolk

Performance with Complex Matrices

To test the chromatographic and mass acquisition methods with matrix samples, a number of challenging sample types were prepared. The TSQ Quantum GC demonstrated excellent sensitivity, selectivity and robustness with these samples, as shown in Figures 4 through 7. These results allow for comparison of the results achieved for the pentachloro-PCBs in matrices covering blood, milk, egg yolk and green cabbage. The TSQ Quantum GC provided clean and background-free mass traces for all types of matrix studied. This selectivity is particularly evident when comparing the matrix samples to the standard samples shown in Figures 1 through 3. Even in very complex samples such as blood (Figure 4) and green cabbage (Figure 7), no increase in the level of background can be observed.

Compared to the standard runs, the PCB concentrations in sample range from a mid-femtogram (fg) to the low picogram (pg) level. PCB concentrations were measured at 0.2 and 1.0 pg/µL for native PCBs and at 100 pg/µL for all added ^{13}C-labeled internal standards. The selectivity of the TSQ Quantum GC virtually eliminates matrix interference, allowing for low detection limits, enhanced confidence in quantitative results, and accurate identification of these compounds.

Conclusion

The Thermo Scientific TSQ Quantum GC facilitates the screening and quantitation of PCBs at low levels in difficult matrix samples and provides results with high certainty. The analytical setup complies with USEPA Method 1668A, following an isotope dilution quantitation protocol. The added ^{13}C-labeled internal standard components were detected with high reliability as demonstrated in different samples with complex matrix background.

Confirmatory methods provide information on the chemical structure of the analyte. The TSQ Quantum GC with its unique hyperbolic quadrupole technology offers superior and uniform selectivity for low level PCB samples in different complex matrices including egg, milk, cabbage and blood. Using the TSQ Quantum GC in H-SRM mode, the PCB pattern that is typical when using high resolution mass spectrometry, such as magnetic sector, can be detected.

The proposed MS/MS measurement scheme using two precursor ions and SRM detection of individual product ions is a valuable solution for screening for PCBs in various complex matrices at the relevant levels. For the fast control of food samples, GC-MS/MS with the TSQ Quantum GC exceeds the current EU directives for a minimum of four (4) identification points, in that the method described here offers five (5) identification points.

For contract and governmental control labs, the TSQ Quantum GC provides a high productivity solution with increased sample throughput even for complex matrix samples. The TSQ Quantum GC delivers ultimate performance in PCB trace analysis with the added economic advantage of using reduced clean-up methods.

References

1. General information about PCBs, see *www.wikipedia.org*
2. Fiedler, H., Polychlorinated Biphenyls (PCBs): Uses and Environmental Releases, UNEP Persistent Organic Pollutants. *www.chem.unep.ch/pops/POPs_Inc/proceedings/bangkok/FIEDLER1.html*
3. Turner, W.E.; Welch, S.M.; et al., Instrumental approaches for improving the detection limit for selected PCDD congeners in samples from the general U.S. population as background levels continue to decline, Proceedings of the Dioxin Conference, Oslo 2006.
4. Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, United States Environmental Protection Agency, Office of Water, EPA No. EPA-821-R-00-002, December 1999
5. EU Commission Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, 12 August 2002, see *http://eur-lex.europa.eu*

Note

The following abbreviations were used in this application note:

MoCB = Monochlorobiphenyl
DiCB = Dichlorobiphenyl
TrCB = Trichlorobiphenyl
TeCB = Tetrachlorobiphenyl
PeCB = Pentachlorobiphenyl
HxCB = Hexachlorobiphenyl
HpCB = Heptachlorobiphenyl
OcCB = Octachlorobiphenyl
NoCB = Nonachlorobiphenyl
DeCB = Decachlorobiphenyl

A suffix "L" following the congener number indicates a labeled compound.

Segment	1	2	3	4	5	6
Duration (min)	9.85	2.60	3.30	4.33	6.03	5.89
Start Time (min)	0	9.85	12.65	15.75	20.08	26.11

Table 4: TSQ Quantum GC H-SRM analysis segments

Segment 1:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	188.04	153.04	0.002	0.150
	190.04	153.04	0.002	0.150
	200.08	165.10	0.002	0.030
	202.08	165.10	0.002	0.030

Segment 2:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	222.00	152.06	0.002	0.080
	224.00	152.06	0.002	0.080
	234.04	164.10	0.002	0.030
	236.04	164.10	0.002	0.030
	255.96	186.02	0.002	0.080
	257.96	186.02	0.002	0.080
	268.00	198.02	0.002	0.030
	270.00	198.02	0.002	0.030

Segment 3:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	255.96	186.02	0.002	0.080
	257.96	186.02	0.002	0.080
	268.00	198.02	0.002	0.030
	270.00	198.02	0.002	0.030
	289.92	219.98	0.002	0.080
	291.92	219.98	0.002	0.080
	301.96	232.02	0.002	0.030
	303.96	232.02	0.002	0.030
	323.90	253.95	0.002	0.080
	325.90	255.95	0.002	0.080
	335.92	265.99	0.002	0.030
	337.92	267.99	0.002	0.030

Segment 4:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	289.92	219.98	0.002	0.080
	291.92	219.98	0.002	0.080
	301.96	232.02	0.002	0.030
	303.96	232.02	0.002	0.030
	323.90	253.95	0.002	0.080
	325.90	255.95	0.002	0.080
	335.92	265.99	0.002	0.030
	337.92	267.99	0.002	0.030
	357.80	287.90	0.002	0.080
	359.80	289.90	0.002	0.080
	369.90	299.95	0.002	0.030
	371.90	301.95	0.002	0.030

Segment 5:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	323.90	253.95	0.002	0.070
	325.90	255.95	0.002	0.070
	335.92	265.99	0.002	0.030
	337.92	267.99	0.002	0.030
	357.80	287.90	0.002	0.070
	359.80	289.90	0.002	0.070
	369.90	299.95	0.002	0.030
	371.90	301.95	0.002	0.030
	391.80	321.90	0.002	0.070
	393.80	323.90	0.002	0.070
	403.80	333.90	0.002	0.030
	405.80	335.90	0.002	0.030
	427.80	357.80	0.002	0.070
	429.80	357.80	0.002	0.070
	439.80	369.90	0.002	0.030
	441.80	369.90	0.002	0.030

Segment 6:	Precursor (*m/z*)	Product (*m/z*)	Width (*m/z*)	Time (s)
	391.80	321.90	0.002	0.070
	393.80	323.90	0.002	0.070
	403.80	333.90	0.002	0.030
	405.80	335.90	0.002	0.030
	427.80	357.80	0.002	0.070
	429.80	357.80	0.002	0.070
	439.80	369.90	0.002	0.030
	441.80	369.90	0.002	0.030
	461.70	391.80	0.002	0.070
	463.70	393.80	0.002	0.070
	473.80	403.80	0.002	0.030
	475.80	405.80	0.002	0.030
	495.70	425.80	0.002	0.070
	497.70	427.80	0.002	0.070
	507.70	437.80	0.002	0.030
	509.70	439.80	0.002	0.030

Table 5: Individual SRM descriptors for acquisition segments 1 through 6

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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Denmark
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