

Screening Foodstuffs for Pesticides and Other Organic Chemical Contaminants Using Full Scan GC/MS and MassHunter Quant Target Deconvolution

Application Note

Food Safety

Author

Chris Sandy
Agilent Technologies UK Ltd
610 Wharfedale Road
Winnersh Triangle
Wokingham, Berkshire
RG41 5TP
UK

Abstract

This application note demonstrates the use of the Target Deconvolution (TD) feature of Agilent MassHunter Quantitative software for the targeted identification of pesticides and other organic contaminants in extracted foodstuffs. TD uses deconvoluted full mass spectra and reference library mass spectral matching to provide additional confidence in target analyte identification beyond that provided by the use of retention time and ion ratio measurements.



Agilent Technologies

Introduction

The benchtop gas chromatograph/single quadrupole mass spectrometer (GC/MS) instrument has been in use as a routine analytical tool for the identification and quantitation of target analytes for the last 25 years. Over this time period, GC/MS systems have become more sensitive, providing ever lower limits of detection for target analytes in a wide range of sample types ranging from food, environmental, forensic/clinical, and flavor/fragrance.

However, what has not changed using single quadrupole GC/MS systems is the normal practice of identifying target analytes based on retention time, and the ratio of qualifier ions to a single quantification ion. While this method works well for the identification of target analytes in relatively clean chromatograms, complex extracted samples such as those obtained from food or environmental samples often have interfering components from the sample matrix, which can cause false positive or false negative results, and make data review both more labor intensive and less objective.

One way to achieve better selectivity from a unit mass resolution GC/MS system is to additionally employ mass spectral deconvolution and comparison of full, deconvoluted component mass spectra to a reference mass spectral database. The spectra can be qualified using target retention time windows and a library match score. The use of retention time locked acquisition methods increases the confidence in target

analyte identification since analyte retention times are maintained despite GC column maintenance or replacement. This reduces the number of false positives identified in any given sample. The use of deconvoluted component spectra can provide additional confidence in the identification of target analytes beyond that provided by retention time and ion ratio measurements.

In 2004, Agilent released the first version of Deconvolution Reporting Software (DRS). DRS is an add-on software package for the MSD ChemStation that combines the ChemStation, AMDIS, and NIST MS Search programs. This package uses full mass spectral deconvolution and library searching to provide additional confidence in the identification of target analytes in complex matrices. The use of DRS to screen for pesticides and other organic contaminants in foodstuffs has been documented previously [1,2].

Target Deconvolution (TD) is a new, totally integrated feature of MassHunter (MH) Quantitative Software first made available in March 2013 with the release of MH Quant version B.06.00. The objective of MH TD is the same as that of DRS, namely, increasing confidence in the identification of target analytes by matching deconvoluted component spectra with reference spectra in a target MS database. The entire TD process is executed within MH Quant itself and does not require passing of any data/information to third party programs. The workflow for TD within MH Quant is shown in Figure 1.

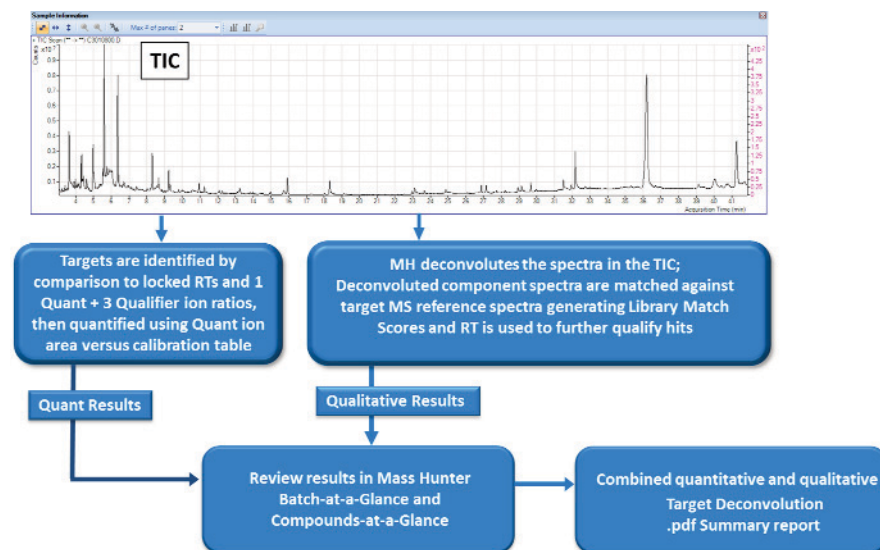


Figure 1. Flow diagram of Target Deconvolution with MassHunter Quant Software.

Experimental

One-microliter hot splitless injections of grape extracts were acquired in full scan mode (40–550 amu) using the original Agilent 1x retention time locked pesticide method. Full GC/MS analysis conditions are given in a previously published Agilent application note [3].

In March 2103, Agilent launched the latest revision of GC/MSD acquisition and data processing software – G1701FA. This version of software uses data acquisition based on the MassHunter platform and generates data files that are compatible with data processing in both the MS ChemStation Data Analysis and MassHunter software packages.

Since the data files in this application note were originally acquired using an earlier revision of MSD ChemStation software, they were converted from ChemStation format to MassHunter format using the provided MH GC/MSD data file translator program. Additionally, the original retention time locked (RTL) pesticide quant method developed for use with the MSD ChemStation, which contains 927 target analytes, was translated to a MH Quant method using the GC/MSD ChemStation Quant Method translator. Both translator programs are installed automatically when MH Quant Software is installed. Screenshots of the translator programs are shown in Figures 2 and 3, respectively.

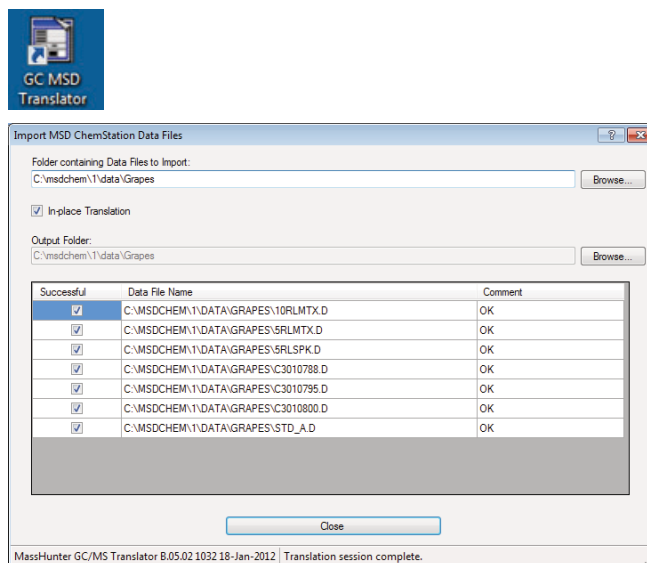


Figure 2. Translating ChemStation data files to MassHunter format.

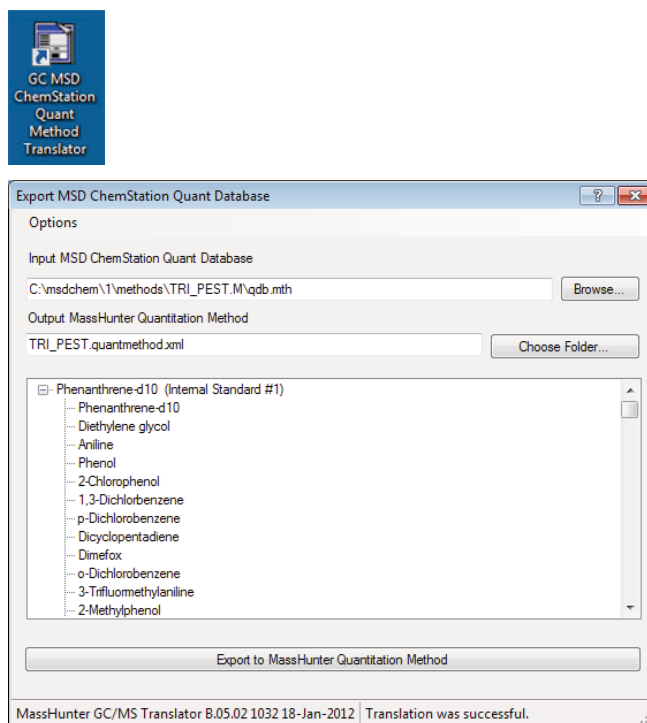


Figure 3. Translating a ChemStation quant method to MassHunter format.

The latest version of the Agilent RTL Pesticide and Endocrine Disruptor MS library (RTLPEST3.L) contains 927 EI mass spectra and their locked retention times. This library was converted for use with the MH Quant method using the built-in functionality of the MH Quant Method editor feature **Set up Reference Library**. This feature compares the target analyte name and CAS number from the MH Quant method calibration table to the same identifiers in the MS library. Matching entries from the .L library are transferred into a MH reference library with the file extension .reflibrary.xml. A screenshot of the reference library creation is shown in Figure 4.

Once the data files, quant method, and MS reference library had been created, the next step was to configure the key parameters of the MH Quant method for use with Target Deconvolution. The parameters used in the grape extract analyses are shown in Table 1.

The spectrum extraction override parameter instructs the MH Quant SW to use the deconvoluted component spectra for matching against the reference MS library. The minimum Library Match Score (LMS) outlier defines the minimum acceptable value, out of 100, from the comparison of the deconvoluted component spectra to the corresponding spectrum in the reference MS library. Components with LMS below this value will not be reported in the final TD summary report. The Library Search Pure Weight Factor is set by default to a value of 0.7 and has been found to be the most appropriate setting for use with TD.

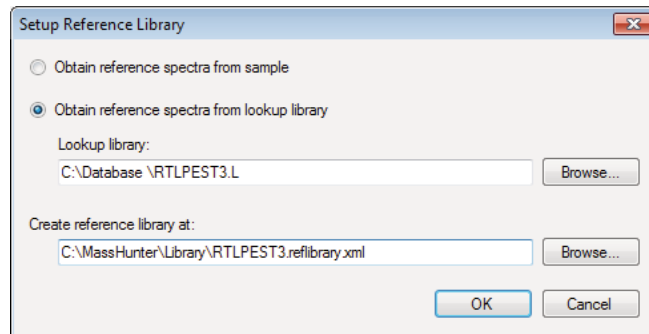


Figure 4. Creation of MH reference mass spectral library.

Table 1. Key MH Quant Method Parameters for Target Deconvolution

MH Quant method parameter	Setting
MH Quant software revision	B.06.00
EIC left/right extraction window	0.25/0.25 minutes
Non reference window	0.33 minutes
Integrator	Agile
Smoothing	None
Spectrum extraction override	Deconvoluted scan
Minimum library match score	45
Minimum purity	50%
Library search pure weight factor	0.7

MassHunter Quant data review with target deconvolution

An example total ion chromatogram (TIC) from a grape extract acquired in full scan mode is shown in Figure 5.

The two main data processing programs within MH Quant SW are Batch at a Glance (BAG) and Compounds at a Glance (CAG). Both programs provide powerful data review capabilities from a totally customizable user interface. BAG gives an overview of all the data files processed within a given batch and results can be viewed sample-by-sample or compound-by-compound in a totally linked environment. Data outliers

may also be applied within BAG and CAG, this alerts the operator to noncompliant results, that is, those below a given value are colored in blue, those above, in red. Applying outliers in BAG and CAG speeds the data review process since the operator need not spend time on reviewing individual analyte results for the targets not clearly identified in the samples. When TD is part of the MH Quant method, two additional columns of data can be displayed in the batch table, namely Library Match Score and Purity.

A screenshot of a typical BAG view from MH Quant SW is shown in Figure 6.

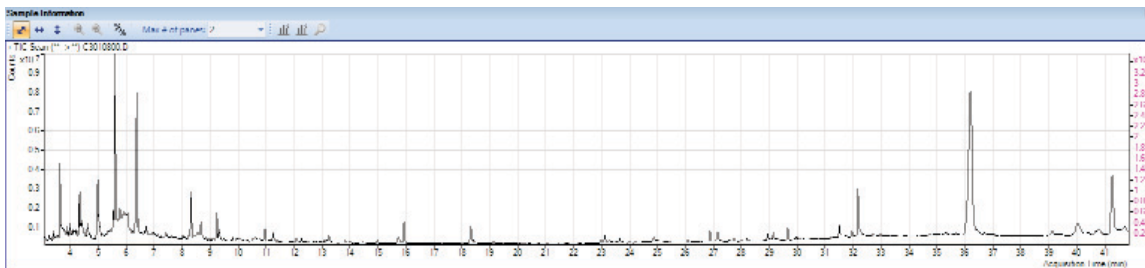


Figure 5. Grape extract TIC.

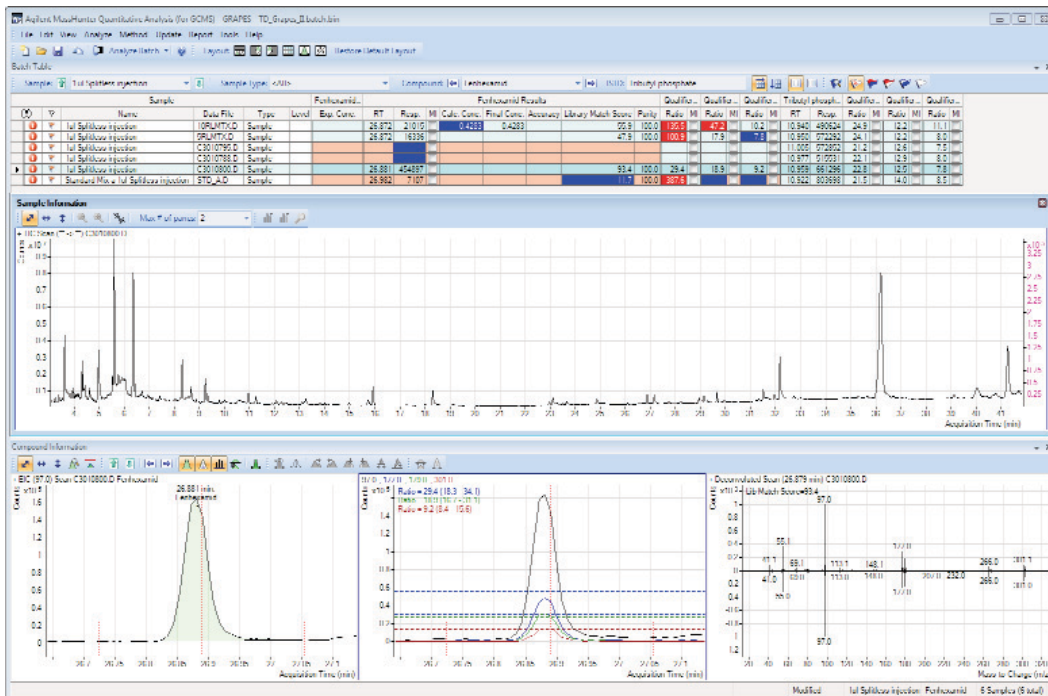


Figure 6. MH Quant SW Batch at a Glance view.

An example Compound Information panel from BAG is shown in Figure 7. The three windows displayed are particularly valuable because they show the details of how an individual target component was identified/quantified. The left panel in Figure 7 shows the integration of the quant ion and how the retention time of the identified peak relates to the expected retention time in the calibration table and to the expected identification window - these are denoted by the vertical red

bars. The center panel shows an overlay of the quant and qualifier extracted ion chromatograms, including the acceptance ranges for the ion ratios as denoted by the horizontal colored bars. The right panel displays the deconvoluted component mass spectrum (top) and, mirrored to it, the spectrum from the MS reference library. This panel also shows the LMS in the top left corner.

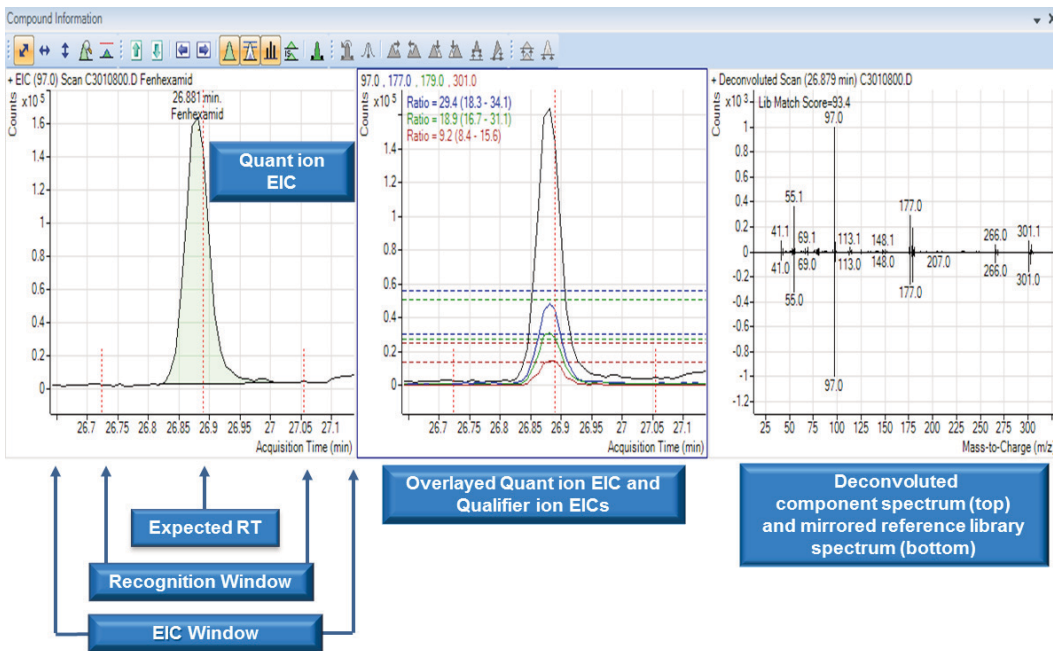


Figure 7. BAG Compound Information including results from Target Deconvolution.

The CAG component of MH Quant SW is another powerful tool for interactive data review since it can be completely customized to suit the preferences of each individual operator. CAG can present any data files from a given batch in column or row format and display any individual target analyte ion chromatograms. Applying outliers to data review in CAG results in target analytes failing one or more outlier tests being highlighted. Typical MH outliers applied to data processed using TD are shown in Figure 8.

An example screenshot of CAG showing the results for 25 of the 927 target analytes in a grape extract sample is shown in Figure 9. Target analytes that fail one or more of the applied outliers are shaded in red. Individual target ion chromatograms may also be manually integrated from within the CAG view by double-left clicking the mouse button on any panel. Manual integrations performed within CAG are linked to the results displayed in BAG.

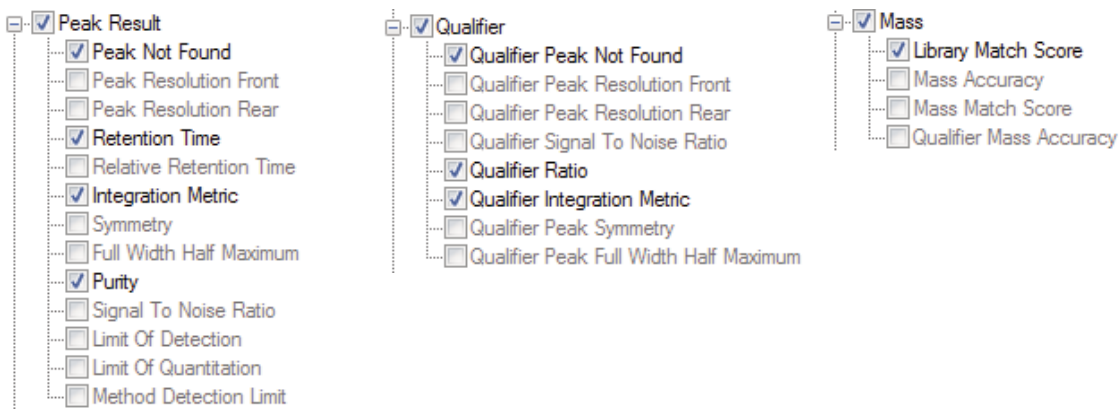


Figure 8. MH Outliers being applied to CAG.



Figure 9. Compounds-at-a-Glance view. Target analytes failing one or more outlier tests are shaded in red.

Reporting target deconvolution results in MH Quant

MH Quant revision B.06.00 is supplied with a customized .pdf report template that will produce summary Target Deconvolution reports. The .pdf template can be found in the \MassHunter\Report Templates\Quant\PDF-Reporting folder and is named TargetedDeconvolution.report.xml. Applying this report to a batch of data employing TD will produce summary reports – typically taking only a few seconds to produce each

TD report. An example TD summary report is shown in Figure 10. The report lists those target analytes which possess Library Match Scores and Purity values greater than the minimum that was set in the MH Quant Method outliers section. The Purity value corrects the final quantitative result in the event of another deconvoluted component with the same m/z as the quantifier ion interfering with the peak area of the reported target component. Thus, a Purity value of 100 denotes that no interference was detected.


Targeted Deconvolution Report							
Sample Name:	1ul Splitless injection						
Data File:	C3010800.D						
Quant Batch Name:	C:\Users\csandy\Desktop\LGC Grapes RTL 1x ESTD\GRAPES\QuantResults\TD_Grapes_II.batch.bin						
Last Calib Update:	7/18/2013 7:17:48 AM						
R.T.	Cas #	Compound Name	Amount/Conc	LMS	R.T. Diff(sec)	Purity	
3.5532	108-95-2	Phenol	0.7064	79	0.3	100.0	
4.3425	95-48-7	2-Methylphenol	0.0449	60	5.8	100.0	
4.3792	106-44-5	4-Methylphenol	0.2901	73	-2.2	90.4	
4.3792	108-39-4	m-Cresol	0.2939	70	-2.6	91.6	
6.3705	003228-03-3	Promecarb artifact [5-isopropyl-3-methylphenol]	2.1476	53	1.6	100.0	
7.6094	30560-19-1	Acephate	1.9987	48	-4.8	100.0	
7.8756	131-11-3	Dimethylphthalate	0.0915	53	-1.8	100.0	
7.9582	85-41-6	Phthalimide	0.0609	66	-1.3	100.0	
8.5730	33704-61-9	Cashmeran	0.8917	63	-2.8	96.1	
8.7565	90-43-7	o-Phenylphenol	0.1026	62	-1.5	100.0	
9.9587	84-66-2	Diethyl phthalate	0.4671	88	0.0	100.0	
13.4184	115-96-8	Tris(2-chloroethyl) phosphate	0.0524	54	0.2	100.0	
13.7947	85-01-8	Phenanthrene	0.0914	58	-1.6	100.0	
14.1709	53112-28-0	Pyrimethanil	0.0960	50	2.6	100.0	
15.9329	84-69-5	Diisobutyl phthalate	27.8507	96	2.3	98.8	
17.4195	111246-15-2	Fipronil, Desulfinyl-	0.1065	60	1.8	100.0	
18.4107	84-74-2	Di-n-butylphthalate	3.3477	94	-0.3	100.0	
21.8153	120068-37-3	Fipronil	0.1320	67	0.3	100.0	
24.7978	120068-36-2	Fipronil-sulfone	0.0298	46	2.7	100.0	
26.5322	58810-48-3	Ofurace	0.1045	56	-2.0	100.0	
26.8809	126833-17-8	Fenhexamid	6.8789	93	-0.5	100.0	
29.6615	117-81-7	Bis(2-ethylhexyl)phthalate	6.8620	95	0.8	100.0	
31.6805	117-84-0	Di-n-octyl phthalate	0.1157	48	0.4	100.0	
36.6911	131860-33-8	Azoxystrobin	1.6227	82	3.7	100.0	

Figure 10. Example .pdf Target Deconvolution report for a grape extract sample.

Results and Discussion

The benchtop gas chromatograph/single quadrupole mass spectrometer (GC/MS) instrument has been in use as a routine analytical tool for the identification and quantitation of target analytes for the last 25 years.

Target analyte identification based on retention time and the ratios of qualifiers ions to a quantifier ion works well in clean samples. However, extracts of food or environmental samples often contain large amounts of co-extracted components that overlap or coelute with the target analytes. In these cases, ion ratios can lead to many false positive and false negative results and rely upon the operator to conduct a detailed and often time-consuming data review process.

The Target Deconvolution (TD) feature of MassHunter Quant Software, introduced with Revision B.06.00 in March 2013, provides additional confidence in target analyte identification beyond the use of retention time and ion ratios. TD is fully integrated into MH Quant SW and can be used to great advantage in the powerful data review programs BAG and CAG through the application of MH outliers during the data review process.

MH Quant B.06.00 includes a predesigned .pdf report template that facilitates the production of TD reports for a single sample, selected samples, or an entire batch.

The seamless integration of TD into MH Quant software provides more confidence in the identification of target analytes without the need to export data/information to third party programs. This ensures that TD is easy to set up, customize, and use, making a significant contribution to improve the productivity of GC/MS systems performing the quantitation of target analytes.

References

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