SHIMADZU
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Technical Report

Screening and Individual Quantitative Works of Regulated Substances in Food Using Triple Quadrupole Mass Spectrometer LCMS-8030

1. Introduction

Our environment is overflowing with a variety of chemical substances, and the effect of these substances on our health is becoming a matter of worldwide concern. Looking at agrochemicals alone, more than a thousand different types of compounds are widely used, therefore it is significantly important to develop a screening method capable of analyzing large number of compounds at low concentration levels. The LC/MS/MS is widely employed for the food safety because it features excellent selectivity and sensitivity by using Multiple Reaction Monitoring (MRM). However, it is well-known that the number of compounds which can be monitored is limited by the length of dwell and pause time because the length of both parameters affects the MRM sensitivity.

The dwell time is the time during which an ion is monitored by the detector in MRM, and the pause time is the time required for MRM switching. Since the dwell time and pause time are parameters which affect the sensitivity of the method as well as the instrument's data sampling rate, the dwell and pause time is a critical parameter in achieving appropriate sensitivity to quantify the regulated substances in a complex food matrix. The LCMS-8030 features extremely fast pause and dwell times of 1 msec.

In this Technical Report, we used the LCMS-8030 triple quadrupole mass spectrometer (Fig. 1) to evaluate whether or not 10 pesticide compounds used to spike a matrix at 10 ppb each could be detected automatically by using generic peak detection method in the screening for 176 pesticides using 1 msec pause and 5 msec dwell times.

Fig. 1 LCMS-8030 Triple Quadrupole Mass Spectrometer

2. Method

A matrix consisting of green tea was used and sample preparation was conducted according to the methods of simultaneous measurement of pesticides using LCMS, specified by Japan's Ministry of Health, Labour and Welfare. The test sample used to evaluate the screening performance consisted of green tea extract solution spiked with 10 pesticides (Carbofuran, Chlorfluazuron, Fosthiazate, Hexathiazox, Indanofan, Lufenuron, Mevinphos, Propoxur, Pyrimidifen, and Tricyclazole), each at a 10 ppb concentration.

A 100 ppb spiked sample was also prepared to develop the generic peak integration parameters used for compound identification. For each MRM mass chromatogram, the largest peak was automatically selected as the target compound to permit automatic identification of target analytes without retention time data. Fig. 2 shows the actual LabSolutions LCMS software (hereafter, LabSolutions) parameters (left: Peak Integration, right: Peak Identification). Of course, the LabSolutions software also supports peak identification using a compound's retention time but the retention time information was not used for peak identification in this experiment.

For the screening, 352 MRM transitions were monitored. Since 2 MRM transitions were set for each compound, 176 compounds could be screened. Of the 176 compounds, the MRM conditions for 167 of these compounds that are included in the Residual Agrochemicals Method Package (Fig. 3) were used.

Since the MRM conditions for each compound can be saved using LabSolutions, multiple MRM transitions can be imported into any LabSolutions HPLC method. Fig. 4 shows the window that is displayed when adding MRM events to the HPLC method (window at left), and the MS event window after the MRM conditions have be imported (window at right). The light blue-colored items are the MRM events in negative mode, and the pink-colored items are the MRM events in positive mode. The width of each band indicates the time window each MRM will be monitored. In this case, all MRM transitions were monitored over the entire chromatographic run, from 0 minutes to 20 minutes.

The dwell and pause time for each MRM was set to 5 msec and 1 msec, respectively. Including polarity switching, the time required for all 352 MRM transitions, including polarity switching, is 2.058 sec. This is called as the Loop Time. Since the chromatographic peak widths are expected to be about 20 sec, approximately 10 data points for each peak will be acquired.

The liquid chromatograph conditions that were used were as follow:
Column: Shim-pack XR-ODS II (75 mmL. × 2.0 mmI.D., 2.2 µm)
Mobile phase A: 5 mmol/L ammonium acetate - water
Mobile phase B: 5 mmol/L ammonium acetate - methanol
Gradient conditions: 30% B (0 min.) → 80% B (4 min.) → 95% B (10 –15 min.) → 30% B (15.01–20 min.)
Flow rate: 0.2 mL/min.
Column oven temperature: 40°C
Injection volume: 2 µL

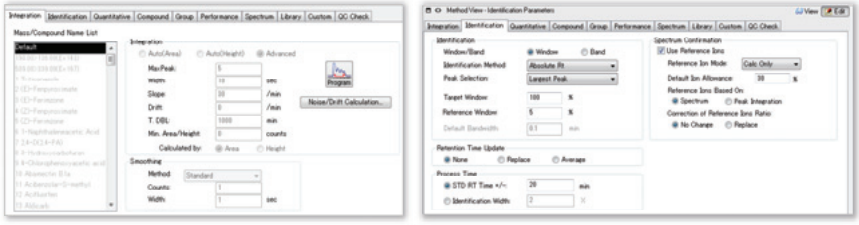

Fig. 2 Peak Integration Parameters (left) and Peak Identification Parameters (right) Screens Used for Screening

- MRM for 167 compounds
- Retention time information
- HPLC conditions

1-Naphthaleneacetic Acid
2,4-D (2,4-PA)
3-Hydroxycarbofuran
4-Chlorophenoxyacetic acid
Abamectin B1a
Acibenzolar-S-methyl
Acifluorfen
Aldicarb
Aldoxycarb (AldicarbSulfone)
Ametryn
Amitraz
Anilofos
Azamethiphos
Azimsulfuron
Azinphos-methyl
Azoxystrobin
Benalaxyl
Bendiocarb
Benfuracarb
Bensulfuron-methyl
Benzofenap
Boscalid
Bromacil
Bromoxynil
Butafenacil
Carbaryl (NAC)
Carbetamide
Carbofuran
Carbosulfan
Carfentrazone-ethyl
Carpropamid
Chlorbufam
Chlorfluazuron
Chloridazon
Chlorimuron-ethyl
Chloroxuron
Chlorsulfuron
Chromafenozide
Cinosulfuron
Clodinafopacid
Clodinafop-propargyl
Clofentezine
Cloprop
Cloquintocet-mexyl
Cloransulam-methyl
Clothianidin
Cumyluron
Cyazofamid
Cycloate
Cycloprothrin
Cyclosulfamuron
Cyflufenamid
Cyprodinil
Dichlorprop
Diclosulam
Diflubenzuron
Dimethirimol
Dimetomorph
Diuron (DCMU)
Dymron (Daimuron)
Epoxiconazole
Ethametsulfuron-methyl
Ethoxysulfuron
Etoxazole
Fenamidone
Fenhexamid
Fenoxaprop-ethyl
Fenoxycarb
Fenpropimorph
(E)-Fenpyroximate
(Z)-Fenpyroximate
(E)-Ferimzone
(Z)-Ferimzone
Flamprop-methyl
Flazasulfuron
Florasuram
Fluazifop
Fluazuron
Flufenacet
Flufenoxuron
Flumetsulam
Fluridone
Fluroxypyr
Fomesafen
Forchlorfenuron
Furametpyr
Furathiocarb
Gibberellin
Halosulfuron-methyl
Haloxyfop
Hexaflumuron
Hexythiazox
Imazalil
Imazosulfuron
Imidacloprid
Indanofan
Indoxacarb
Iodosulfuron-methyl
Ioxynil
Iprovalicarb
Isoxaflutole
Linuron
Lufenuron
MCPA (MCP)
MCPB
Mecoprop (MCPP)
Mefenpyr-diethyl
Mepanipyrim
Mesosulfuron-methyl
Methabenzthiazuron
Methiocarb
Methomyl
Methoxyfenozide
Metosulam
Metsulfuron-methyl
Monolinuron
Naproanilide
Naptalam
Novaluron
Oryzalin
Oxabetrinil
Oxamyl
Oxaziclomefone
Oxycarboxin
Pencycuron
Penoxsulam
Pentoxazone
Phoxim
Pirimicarb
Primisulfuron-methyl
Prometryn
Propaquizafop
Propoxycarbazone
Prosulfuron
Pymetrozine
Pyraclostrobin
Pyrazolynate
Pyrazophos
Pyrazosulfuron-ethyl
Pyriftalid
Pyrimethanil
Quizalofop-ethyl
Silafluofen
Simeconazole
SpinosynA
SpinosynD
Sulfosulfuron
Tebufenozide
Tebuthiuron
Teflubenzuron
Tetrachlorvinphos (CVMP)
Thiabendazole
Thiacloprid
Thiamethoxam
Thidiazuron
Thifensulfuron-methyl
Thiodicarb
Triasulfuron
Tribenuron-methyl
Triclopyr
Trifloxystrobin
Trifloxysulfuron
Triflumizole
TriflumizoleMetabolite
Triflumuron
Triflusulfuron-methyl
Triticonazole

Analytical column : Shim-pack FC-ODS (2.0 mmI.D. × 150 mmL., 3 µm)
Mobile phase A : 5 mmol/L ammonium acetate - Water
Mobile phase B : 5 mmol/L ammonium acetate - Methanol
Flow rate : 0.2 mL / min.
Injection volume : 1 µL

Step	Time (min.)	A (%)	B (%)	Step	Time (min.)	A (%)	B (%)
0	0	85	15	5	17.5	5	95
1	1	60	40	6	30	5	95
2	3.5	60	40	7	30.1	85	15
3	6	50	50	8	40	85	15
4	8	45	55				

Fig. 3 Residual Agrochemicals LC/MS/MS Method Package

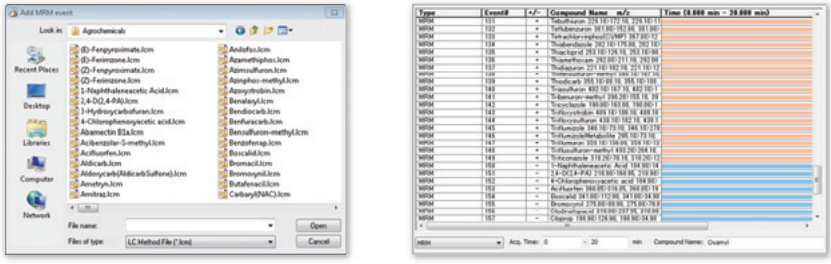

Fig. 4 Adding MRM Events (left), Analysis Screen After Loading MRM Conditions (right)
Light blue-colored items are negative MRM, and pink-colored items are positive MRM.
The width of band indicates the monitoring time span.

3. Screening Results for Green Tea Matrix

Fig. 5 shows the detection results for a sample consisting of green tea spiked with 10 pesticides, each at a concentration of 100 ppb. In the data processing window of LabSolutions, the respective tables and chromatograms are linked, and show the pesticide chromatogram associated with the corresponding green-colored row in the table. The ▼ at the peak center is the peak detection mark, whose retention time is displayed in the table. The red up arrow and down arrow indicate the peak start point and end point, respectively. The "Auto" in the Mode column of the table indicates that the peak was automatically detected. Compounds which were not detected display the phrase "No peak is detected."

In the Mevinphos chromatogram (second from top chromatogram at right in Fig. 5), 3 peaks are detected including Mevinphos, but the

correct peak was identified because the largest peak was specified in the peak identification conditions. The remaining 9 pesticides were also correctly identified.

The detection results for green tea extraction spiked with the 10 pesticides at a concentration of 10 ppb each are also displayed in Fig. 6. Seven of the ten pesticides were detected and identified, while Chlorfluazuron, Indanofan, and Lufenuron were detected but not automatically identified. It was clear that the dwell time of 5 msec was too short for detection and automatic identification of 10 substances present at 10 ppb without retention time information, but all 10 substances were identified when retention time information was used.

Fig. 7 shows the chromatograms of the 10 pesticides measured using a dwell time of 20 msec. The 3 substances (Chlorfluazuron, Indanofan, Lufenuron) that were not detected in the screening with generic peak detection parameters were detected without using retention time information.

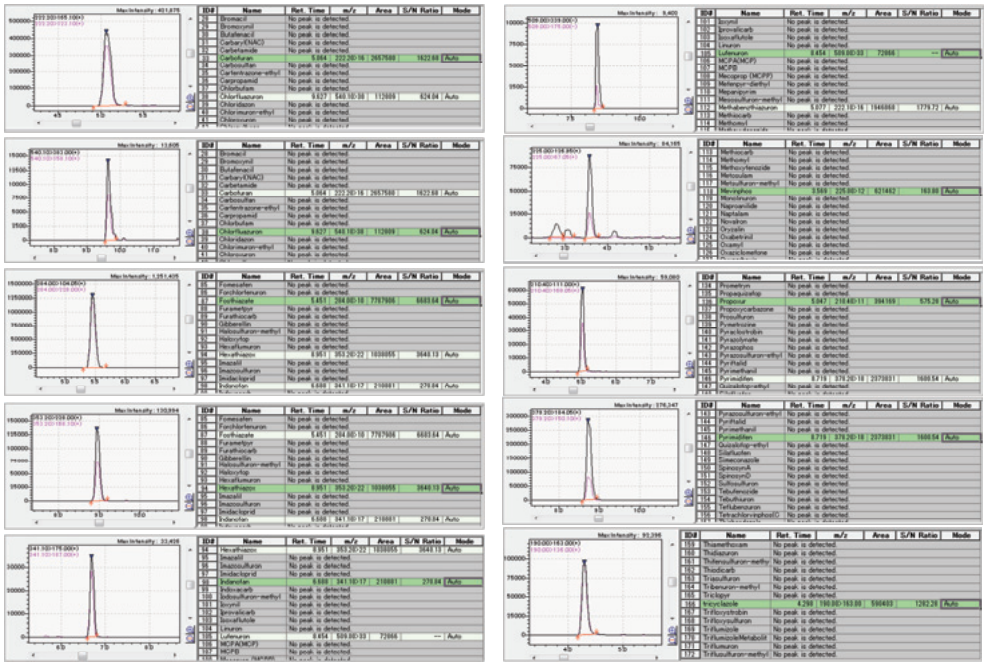

Fig. 5 Detection Results for 100 ppb-Spiked Sample (Green Tea)
"Auto" in the Mode column indicates peaks that were detected automatically.

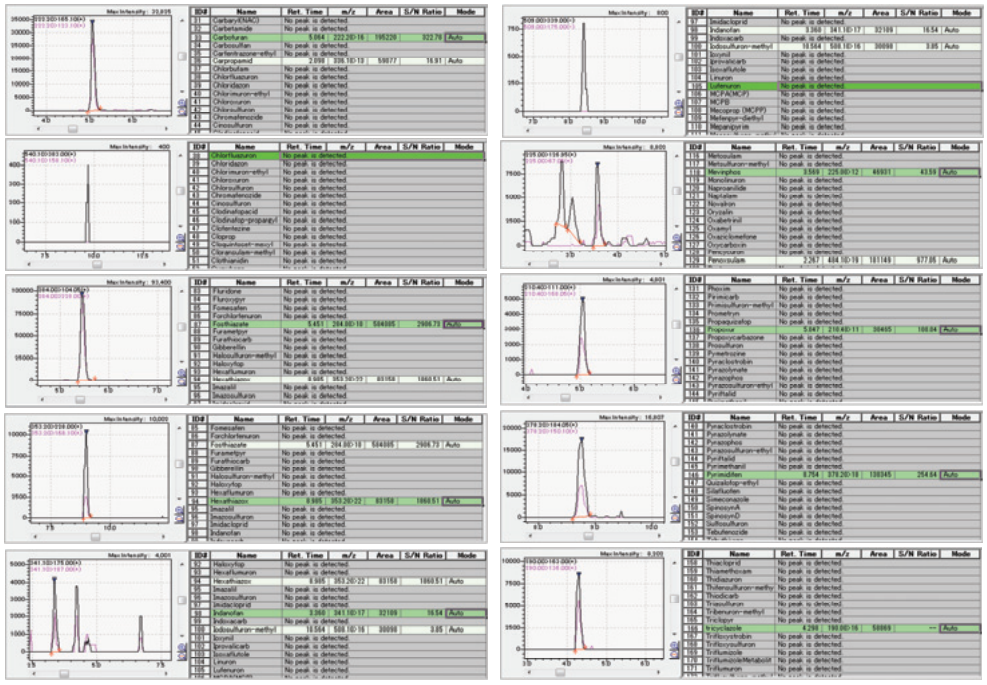

Fig. 6 Detection Results for 10 ppb-Spiked Sample (Green Tea)

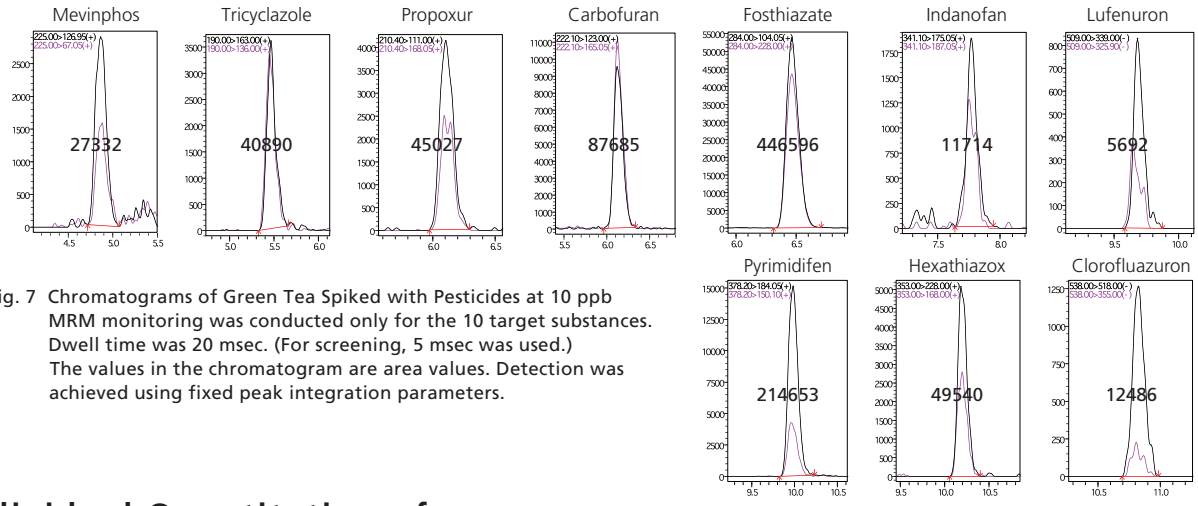

Fig. 7 Chromatograms of Green Tea Spiked with Pesticides at 10 ppb
MRM monitoring was conducted only for the 10 target substances.
Dwell time was 20 msec. (For screening, 5 msec was used.)
The values in the chromatogram are area values. Detection was achieved using fixed peak integration parameters.

4. Individual Quantitation of 10 Pesticides in Green Tea

Due to the excessive presence of caffeine in the green tea matrix, it was necessary to assess the influence of caffeine on the quantitation values of the various pesticides. When analysis was conducted on the matrix-spiked sample, the area values of the respective pesticides showed great variation. For example, after a 100 ppb spiked sample was injected in the midst of injecting a 100 ppb standard, the %RSD ranged from 5.4% (Tricyclazole) to 37.3% (Chlorfluazuron). To suppress the influence of caffeine, a divert valve was inserted between the column outlet and the MS to eliminate the caffeine. As a result, the area value %RSD of each pesticide in the samples spiked at 10 ppb was less than 10%.

There was no attempt during screening to increase the sensitivity by optimizing the ionization parameters, but here, the ionization parameters were optimized in order to obtain better sensitivity. Table 1 shows the details of the optimized parameters. Fig. 8 shows the chromatograms of Propoxur, Carbofuran, Indanofan and Hexathiazox obtained before and after optimization of the ionization parameters. With the pesticide standards, the area values of the pesticides increased about 2-fold as a result of optimizing the ionization parameters.

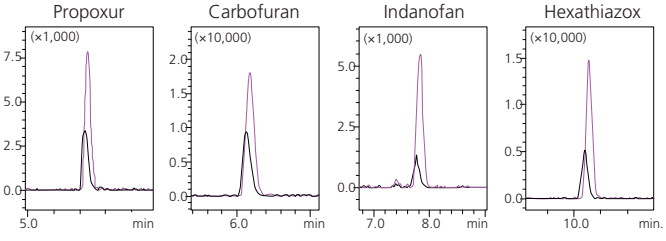

Fig. 8 Comparison of Chromatograms Before and After Optimization of Ionization Parameters

Table 1 Optimization of Ionization Parameters

Nebulizing Gas Flow	1.5 L / min. → 3.0 L / min.
Drying Gas Flow	10 L / min.
DL Temperature	250°C → 300°C
BH Temperature	400°C → 500°C
Probe Position	+2 → +3

5. Conclusion

As shown in Fig. 7, a 20 msec dwell time was best for detection and automatic identification of the pesticides added to green tea at 10 ppb. If the minimum dwell time that satisfies the target quantitation limit can be determined, the number of compounds that can be screened in a single measurement will depend on the HPLC method that will be used and the instrument's polarity switching speed. Since polarity switching takes only 15 msec with the LCMS-8030, and assuming that 10 sampling points can be acquired for each substance due to the approximately 20 sec required for elution of each peak based on the employed HPLC conditions, 100 compounds can be screened and quantitated using a dwell time setting of 20 msec and limiting the number of monitored MRMs to one per compound.

By using the retention time information, each of the 10 analyte pesticides could be detected at a concentration of 10 ppb in green tea, even with the extremely short dwell time of 5 msec. Simultaneous analysis methods, which also include HPLC conditions, have been published by each country's regulating authority. The sensitivity and speed of the LCMS-8030 more than satisfy these requirements for multi-component screening and quantitation.

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