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應用手冊

Best Practice for the Use of Xevo TQ-S cronos for Residue Analysis in Food – Determination of Triphenylmethane Dyes in Shrimps

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Abstract

This application note presents steps to optimize the electrospray (ESI) source of Xevo TQ-S cronos, a type of tandem quadrupole mass spectrometer, focusing on probe positioning and cone gas flow to achieve the best precision and sensitivity for the determination of malachite green and other dyes in seafood extract. The optimum cone gas flow was found to be close to 0 L/hr (no gas flow) as it provided the lowest %RSD for peak area of leucomalachite green and leucocrystal violet and acceptable values for the rest of the dyes included in the method. The probe was tested for two positions within the orthogonal geometry of the source. The optimum position furthest from the cone provided the best sensitivity with the lowest %RSD for peak area of all analyzed compounds despite the maximum signal which was achieved for leucocrystal violet and leucomalachite green in shrimps when probe with nebulizer set relatively close to the sample cone. The optimized method parameters were used for an evaluation of performance including linearity, matrix effects, and measurement precision along with sensitivity. The data demonstrated suitability of the method including the use of internal standards in the form of labelled analogues of target compounds for reliable quantitation of dyes in shrimps at the "Reference points for action" (RPA) level.

Benefits

- Use of the unique orthogonal geometry of the Xevo TQ-S cronos source can be easily optimized for reliable sensitivity
- QuEChERS is a rapid and easy-to-use means of preparing extracts suitable for the analysis of dyes in shrimps

Introduction

Triphenylmethane dyes, common commercial and inexpensive fabric dyes, are used illegally in aquaculture for their antimicrobial properties. After digestion, dyes such as malachite green (MG) and crystal violet (CV), are rapidly metabolized to reduced leuco- forms (LMG and LCV) which can persist in fish long after dosing, causing adverse effects in aquatic species and mammals.[1] MG is banned for use in food-producing animals in major geographies according to regulations set by the U.S. Food and Drug Administration (FDA), Codex Alimentarius FAO-WHO, and European Commission. The EU sets the reference point for action (RPA) which has been

established for MG of 0.5 µg/kg (for the sum of MG and LMG) to ensure the functioning of controls for food of animal origin including imports. Food of animal origin containing residues of such substances at or above the RPA is considered non-compliant with Union legislation.[2] Therefore, suitable methods are required to monitor compliance with such regulations.[3]

The Xevo TQ-S cronos instrument has been developed as a reliable system for routine quantitative analysis, incorporating sample cone design elements that have been previously utilized in the widely-used ACQUITY QDa Mass Detector. As part of the reverse cone design, the narrowest restriction is in the center of the cone whereas the entrance to the cone is comparatively wide. This design ensures that sample matrix and mobile phase buffer salts will not aggregate and block the orifice. This increases the uptime of the instrument between cone cleans and provides reliable sensitivity for complex food matrices.

In addition to the reverse cone design, the established technology also contributes towards the robust performance of Xevo TQ-S cronos. The ion source uses a dual orthogonal geometry, which enables efficient transmission of ions into the analyzer at the same time as removing non-ionized materials (neutrals). The StepWave is an off-axis ion guide further removing neutral species and reducing gas load, actively extracting the ion beam into a parallel “off axis” ion tunnel which results in improved transmission enhancing both sensitivity and robustness. The collision cell uses travelling wave technology to reduce the residence time of ions in the collision cell allowing rapid multi component MRM data acquisition without loss in signal intensity while minimizing cross talk between adjacent MRM channels. This ensures full compatibility with the high data acquisition rates required for high-quality multi-component UPLC-MS/MS quantitative analysis.

A method for the determination of triphenylmethane dyes and their metabolites in shrimp was previously reported using the Xevo TQD System, which was shown to be a cost-effective solution for the determination of such banned substances.[4] In this application note we present an UPLC-MS/MS method using the latest Xevo TQ-S cronos instrument for the accurate and precise quantification of dyes and their metabolites, suitable for checking shrimps for compliance with European regulations. The structures of the dyes included in the study are given in Figure 1.

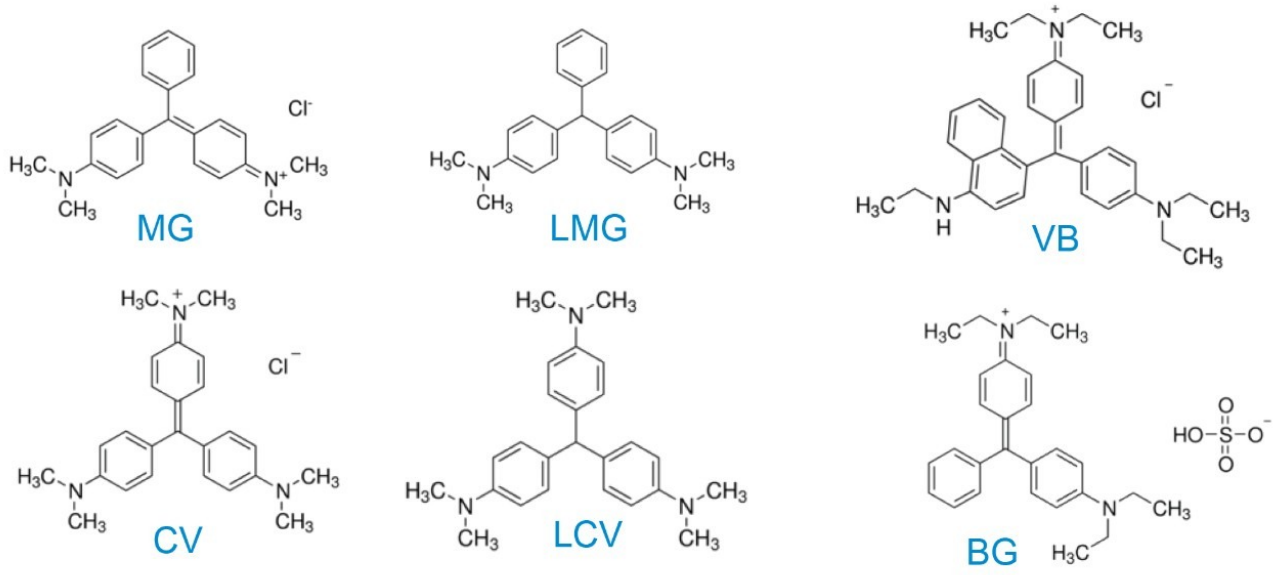

Figure 1. Chemical structures of dyes included in the study.

Experimental

Extraction and Clean-up

A modified version of QuEChERS was used to prepare extracts of shrimps.[4] Briefly, 10 g of homogenized sample was extracted with 10 mL of acetonitrile containing 1% acetic acid and shaken manually for 1 min. A QuEChERS pouch was added (P/N 186006812 <https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/186006812-disque-aoac-method-15-g-sodium-acetate-and-6-g-mgso4-50-ml-pouch.html>) containing 1.5 sodium acetate and 6 g magnesium sulfate and the mixture was placed on an ultrasonic bath for 10 minutes and centrifuged. The supernatant was transferred to a 15 mL DisQuE QuEChERS PSA Tube (P/N 186004833 < https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/186004833-disque-900-mg-mgso4--150-mg-psa-15-ml-tube-50-pk.html>) containing 900 mg magnesium sulfate and 150 mg PSA. The sample was shaken by hand for 1 min, centrifuged and used to prepare the matrix-matched standards at eight concentrations (0.05, 0.1, 0.25, 0.5, 1, 2, 5, 10 ng/mL) equivalent to the range of 0.05 to 10 µg/kg. Stable isotope analogues were added as internal standards after the sample extraction. To stabilize dyes, it is recommended to add antioxidant, such as ascorbic acid (0.05 mM) prior to the storage of samples, or hydroxylamine hydrochloride before the extraction (500 µL of solution at 9.5 g/L).

ESI Source Optimization

To achieve reliable sensitivity, which is defined by peak area and its %RSD over repeated injections of standard prepared in matrix extract, it is necessary to optimize source settings such as the ESI capillary protrusion and applied voltage, desolvation gas flow, and the temperatures of the gas and the source block. It is also important to take into consideration the composition of the mobile phase used for elution of analytes from the UPLC column. Optimization of these parameters has an impact on formation of solvent droplets, charge transfer and formation of ions which are transferred from the atmospheric pressure of the ESI source to the high vacuum of the StepWave ion optics and the MS analyzer.

As part of this investigation into optimizing the sensitivity and repeatability in complex matrix, the two important tunable ESI source parameters were found to be ESI probe position and cone gas flow. The ion source of Xevo TQ-S cronos has dual orthogonal geometry that enables efficient transmission of ions into the analyzer whilst removing non-ionized materials (neutrals). The tunable position (horizontal and vertical) of the ESI probe including nebulizer and ESI capillary using a graded vernier scale enables adjustment to achieve the optimum signal intensity and good precision at the same time. The vernier scale for tested horizontal position 1 and 2 is captured in Figure 2.

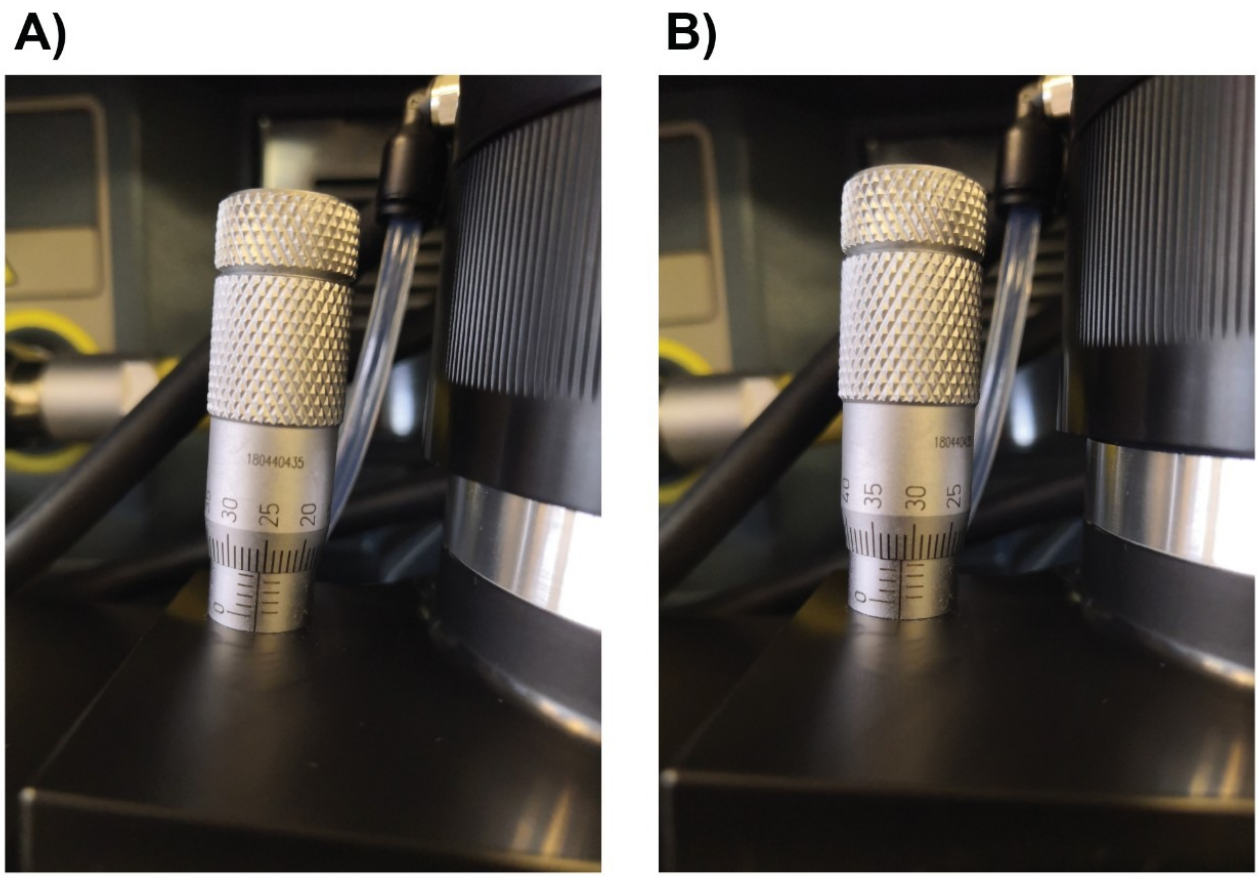

Figure 2. Setting of the vernier scale for the horizontal position of the ESI probe for the analysis of dyes in shrimps. A) position 1 for the analysis of dyes in shrimps B) position 2 for the best signal of leucocrystal violet.

A second parameter that was investigated in this study was the cone gas flow. This stream of nitrogen prevents large droplets from entering the orifice of the sample cone from where the ions are transferred to the MS analyzer. The reduction or prevention of large solvent droplets entering the MS reduces the formation of solvent clusters, e.g. [M + n (H_2O) + H)], which simplifies the interpretation of the generated data. The effects of both probe position and cone gas flow on the response and precision of the determination of the dyes in shrimps extracts have been investigated.

UPLC-MS/MS Conditions

UPLC system:	ACQUITY UPLC I-Class PLUS with FTN Sample Manager
Column:	ACQUITY UPLC BEH C_{18} Column (1.7 µm,

	2.1×100 mm, P/N 186002352)
Mobile phase A:	5 mM Ammonium formate, pH 4.5
Mobile phase B:	0.1 % Formic acid in acetonitrile
Flow rate:	0.25 mL/min
Injection volume:	5 µL
Column temp.:	40 °C
Sample temp.:	10 °C

Gradient

Time	Flow rate	% A	% B	Curve
Initial	0.25	60	40	Initial
1	0.25	10	90	6
6	0.25	10	90	6
7	0.25	60	40	6
11	0.25	60	40	6

MS instrument:	Xevo TQ-S cronos
Ionisation:	Electrospray
Polarity:	Positive
Capillary voltage:	0.3 kV
Desolvation temperature:	600 °C
Desolvation gas flow:	1000 L/hr

Source temperature:	150 °C
Cone gas flow:	0 L/hr

The optimized cone voltage and collision energy for analyzed dyes are summarized in Table 1. A calculation of the MS cycle of the final MRM method in positive mode was automatically optimized using the auto-dwell function in the MassLynx Software with a peak width of 5 s which required 12 points per peak.

Compound name	R. time (min)	Polarity	MRM *m/z* for Q (q)	CV [V]	CE [eV]
Malachite green	1.89	ESI+	329.3 > 208.1 (329.3 > 313.4)	60	30 35
Malachite green picrate d5	1.89		334.2 > 318.2	65	32
Leucomalachite green	2.95		331.3 > 239.2 (331.3 > 316.4)	45	30 20
Leucomalachite green d5	2.93		336.3 > 239.2	35	30
Crystal violet	2.12		372.3 > 251.3 (372.3 > 356.3)	100	25 30
Crystal violet d6 trihydrate	2.11		378.2 > 362.2	65	35
Leucocrystal violet	2.93		374.3 > 238.1 (374.3 > 358.2)	50	28 30
Leucocrystal violet d6	2.89		380.3 > 364.3	40	30
Brilliant green	2.31		385.3 > 297.2 (385.3 > 341.2)	70	50 35
Victoria blue	2.61		478.3 > 329.2 (478.3 > 434.3)	60	35 40

Table 1. Optimized MRM conditions for the detection of triphenylmethane dyes in shrimps.

Results and Discussion

The effect of the ESI probe position on peak area and precision for average peak area (n inj. = 6) of a selection of triphenylmethane dyes in shrimps extract is shown in Figure 3. Normalized peak areas to the sum of each analyte are shown for two of the different horizontal positions of the ESI probe investigated. Although position 1 was the setting that proved optimal for the determination of most of the dyes in shrimps, position 2 was found to give the largest signal for leucocrystal violet. The optimized position of the probe for evaluation of performance of a Xevo TQ-S cronos for the analysis of dyes in shrimps was set to position 1 as it provided two to three times lower %RSD for all compounds while maintaining good peak area for leucocrystal violet and leucomalachite

green to achieve the best sensitivity as these two analytes provide the lowest relative response.

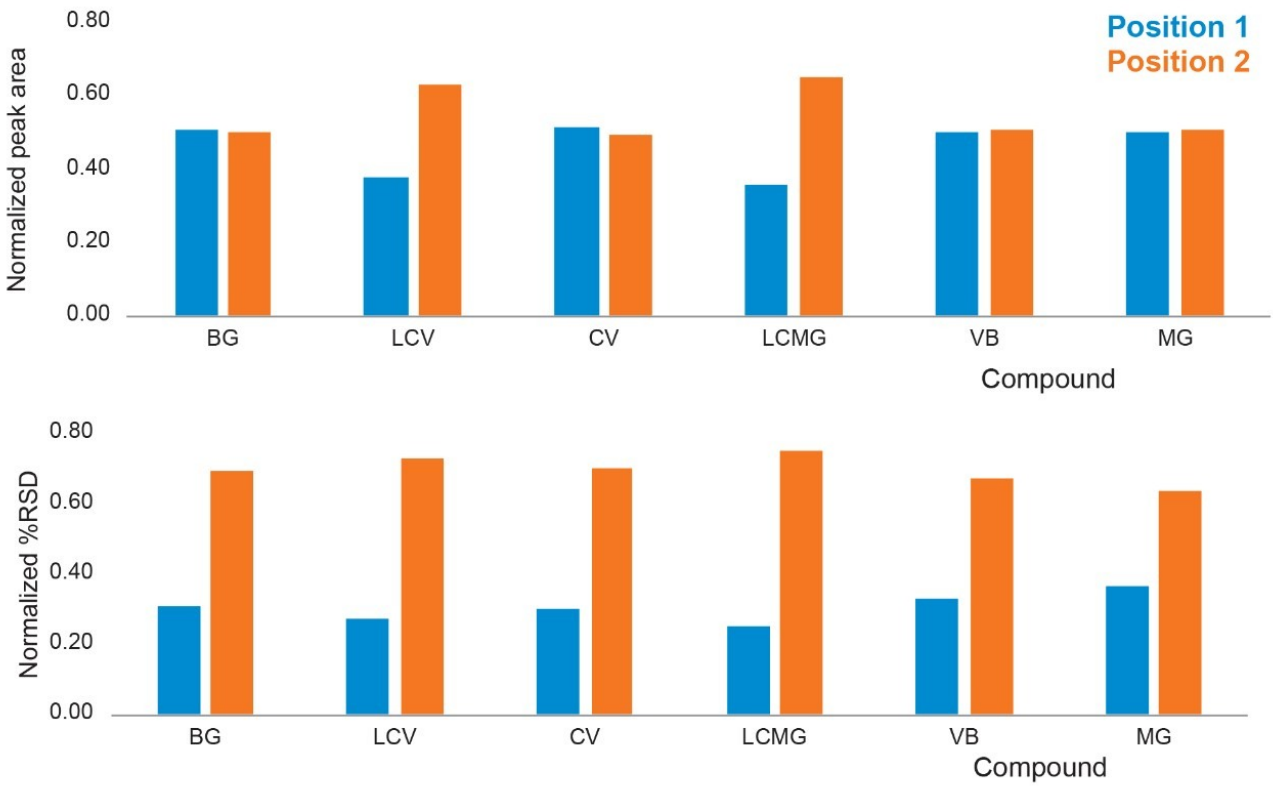

Figure 3. Impact of the ESI probe position on the sensitivity of Xevo TQ-S cronos for triphenylmethane dyes at 0.5 µg/kg in shrimps extract.

The effect of the cone gas flow on the precision for the determination of the dyes in shrimps extract is shown in Figure 4. Setting the cone gas to 0 L/hr was shown to give significantly improved precision for leucomalachite green and leucocrystal violet, whilst retaining acceptable precision for the rest of the compounds of interest. The overall response of compounds was not significantly different across the tested range. Previous experiments on Xevo TQ-S cronos showed that cone gas flow >50 L/hr can reduce the signal.[5] Xevo TQ-S cronos, Xevo TQ-S micro, and Xevo TQD instruments allow cone gas flows to be set between 0 and 300 L/hr. The optimum gas flow rate is determined by the diameter of the orifice of the sampling cone: with increased diameter increases the optimum flow rate. At the same time, a cone gas flow above the experimental optimum can cause significant signal loss. In this case, the signal can be recovered by applying higher capillary voltage which encourages creation of aerosols and subsequently the formation of ions. However, too high capillary voltage can cause undesired reductive or oxidative processes as well as a reduction of the signal intensity due to discharge effects which can be observed as lightening on the capillary. This can occur at values above around 2.3 kV in negative mode and 3.5 kV in positive mode depending on the capillary protrusion. Generally, the discharge effects are

observed at lower voltage when the capillary protrusion is larger.

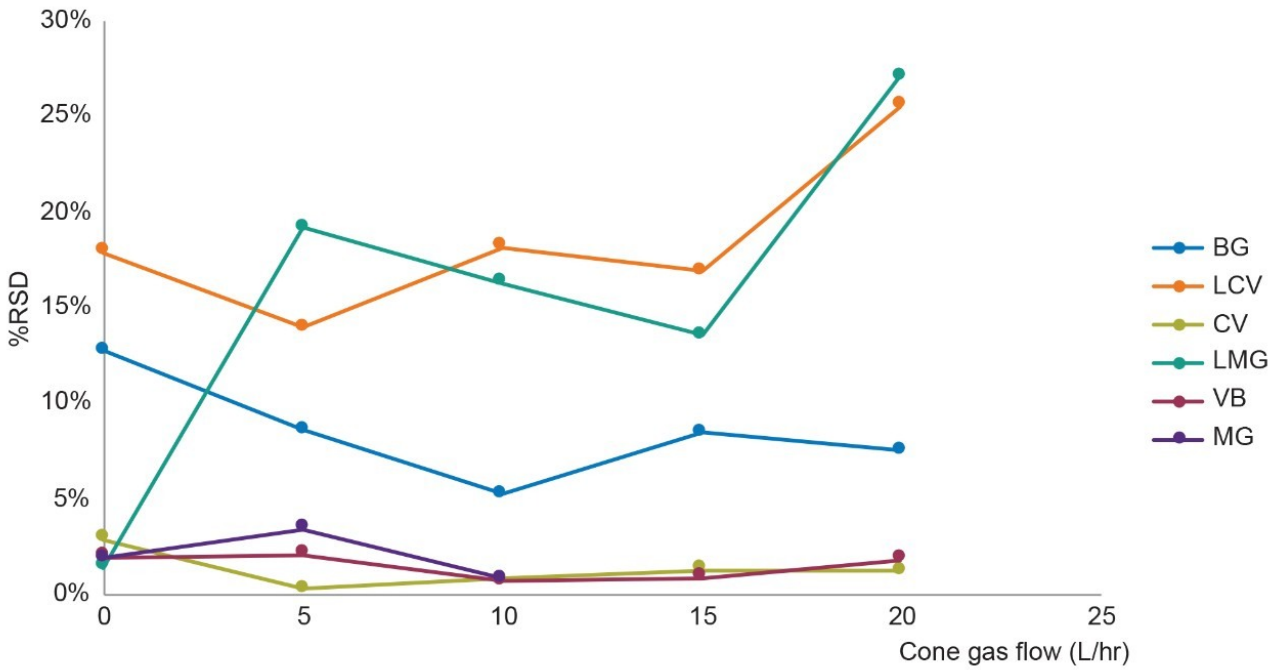

Figure 4. %RSD (n=6) for peak area of dyes in shrimps extracts using different cone gas flows (L/hr).

Linearity and Matrix Effects

For plotting the calibration curves, a weighting factor of 1/x was applied. The linearity was assessed across the whole concentration range and the R^2 factor was found to be greater than 0.99 and residuals expressing deviation of a calculated value from the true value below 20% for most of analytes using the internal standard method.

Assessment of matrix effects (ME), which occur during the ionization process in the MS source should be one of the routine steps during validation of any quantitative ESI-LC-MS method as they can affect both accuracy and sensitivity.[6] For expression of ME for dyes in shrimps was used the ratio of the slope of the calibration curve prepared in matrix and its equivalent in solvent:

$$ME = a_M/a_S$$

where a_M and a_S stands for coefficients expressing the slope of calibration curve in matrix and solvent. The closer the calculated ME is to 1, the lower the matrix effects are. An ME < 1 defines signal suppression, ME > 1 characterizes signal enhancement.

To compensate ME in routine quantitative LC-MS/MS it is possible to generate a matrix matched calibration curve. Another approach that compensates for changes in response due to ME is the use of isotopically labelled

analogues as internal standards. If labelled standards are added before the extraction procedure, they compensate for recovery and ME. The degree of ME then reflects both: The loss of analytes during the extraction from the sample as well as ME which occur during the ionization processes in the ESI source caused by co-eluting molecules. An example of a quantitation of ME is shown in Figure 5 and a summary of measured matrix effects for dyes in shrimps is shown in Table 2. Only LCV exhibited a significant matrix effect, therefore it is necessary to use the internal standard or matrix matched calibration curve.

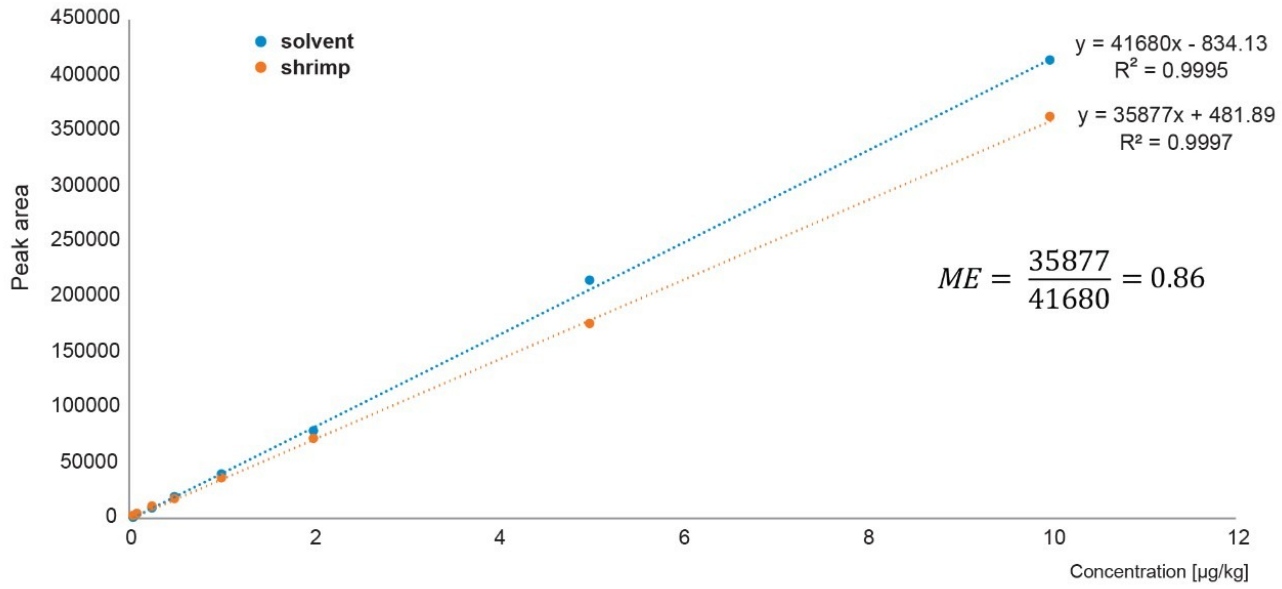

Figure 5. Example of a calculation of matrix effects for crystal violet in extract of shrimps.

Compound	ME
BG	0.77
LCV	10.00
CV	0.86
LCMG	0.20
VB	0.68
MG	0.91

Table 2. Matrix effects measured for target compounds in extract of shrimps.

Measurement Precision and Sensitivity

To investigate the repeatability of the measurements, multiple injections (n = 10) were made of two matrix-matched standards in shrimps (0.5 and 5.0 µg/kg). The calculated %RSD for peak areas of dyes are given in Table 3 and typical chromatograms for all analytes are presented in Figure 6, which shows this method to be suitable for checking regulatory compliance for these dyes in shrimps.

Compound	0.5 µg/kg (n = 10)	5 µg/kg (n = 10)
BG	2.1%	0.9%
LCV	6.1%	3.4%
CV	1.1%	0.5%
LCMG	2.2%	2.6%
VB	1.1%	1.8%
MG	1.8%	0.6%

Table 3. %RSD for peak area of dyes detected with TQ-S cronos in extract of shrimps spiked at 0.5 and 5 µg/kg.

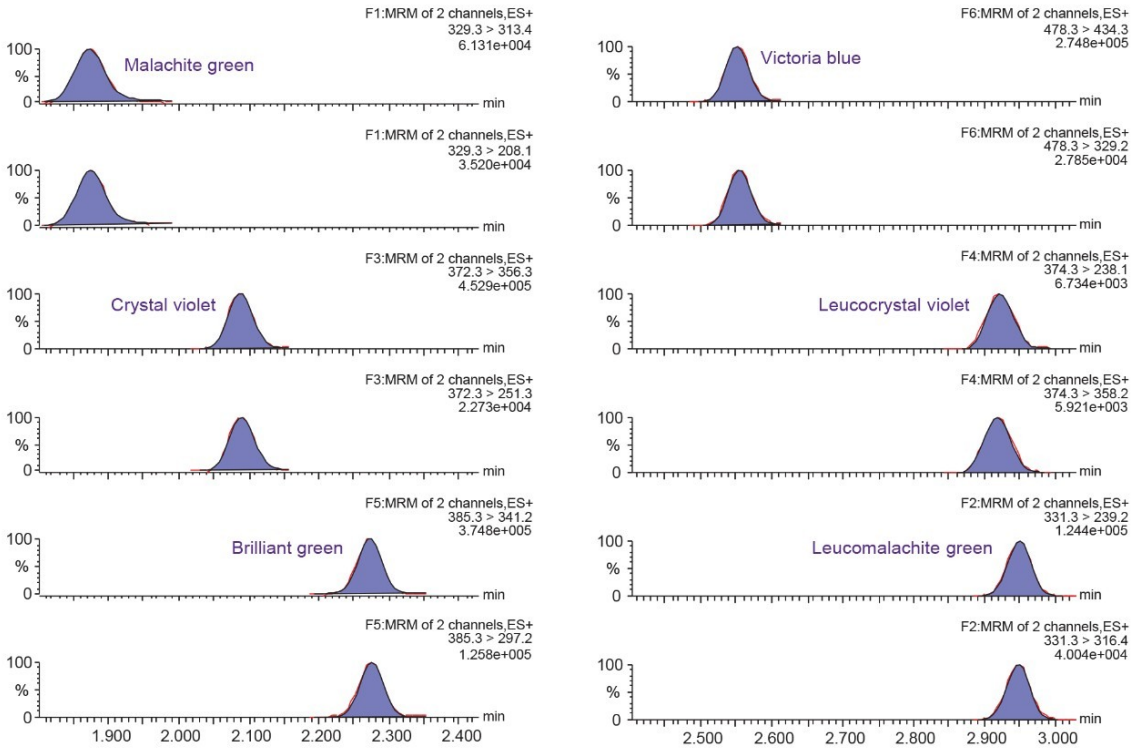

Figure 6. Example of two MRM chromatograms of dyes at 0.5 µg/kg in shrimps.

Conclusion

Triphenylmethane dyes including malachite green are highly toxic compounds which need to be monitored in food of animal origin according to EU regulations. The position of the ESI probe within the orthogonal geometry of the Xevo TQ-S cronos source was adjusted for optimum sensitivity with an %RSD <6% so that malachite green and the other dyes of interest could be detected in shrimps extract at the RPA level. The cone gas flow was optimized to a setting of 0 L/hr to achieve the best balance of highest response and precision. Matrix effects were evaluated and found to be significant for leucocrystal violet. These effects were mitigated by use of matrix-matched standards and the use of isotopically labelled internal standards. This method has shown to be suitable for checking regulatory compliance for these dyes in seafood.

References

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