

# DEVELOPMENT OF UPLC-MSMS METHODS FOR THE ANALYSIS OF COMPLEX, SPARSELY SOLUBLE COMPOUNDS IN ENVIRONMENTAL TOXICOLOGY

---

Jelena (Circic) Jokovic  
Section Head Analytical and Physical Chemistry  
Charles River Laboratories Den Bosch BV



# EVERY STEP OF THE WAY



Founded in 1947 by Dr. Henry Foster



A leading, full-service drug discovery and development company


















Our scientists worked on ~50% of the drugs approved by the FDA in the past 2 years





# INDUSTRIES SERVED

Our mission is to provide exactly what researchers need to develop new therapeutics for patients as rapidly as possible

 ACADEMIC	 PHARMA	 HOME	 CHEMICAL	 FOOD & DAIRY
 GOVERNMENT	 BIOTECH	 BEAUTY	 DIALYSIS	 MED DEVICE
 AGROCHEMICAL	 NUTRACEUTICAL	 NUCLEAR MEDICINE	 COMPOUNDING	 ANIMAL HEALTH

# BIOACCUMULATION IN FISH

## Dietary Exposure, OECD 305

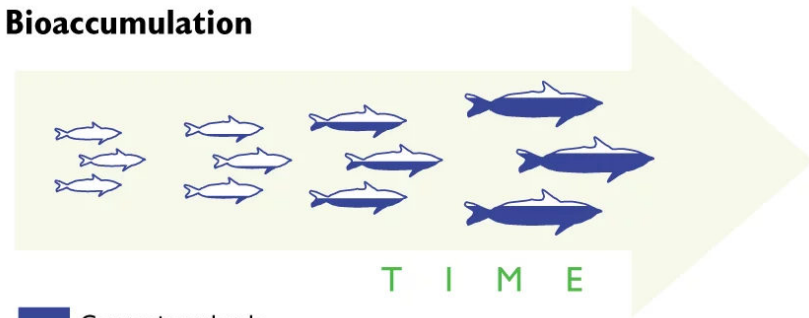
### Persistent toxicants

- Bioaccumulation
- Build up in tissue (fat, muscle, bone)
- Migration in the food chain
- Biomagnification

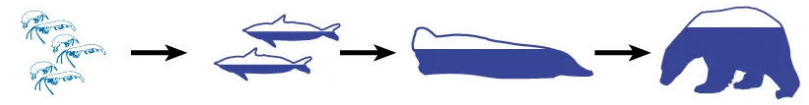
“Fish are exposed to the chemical dissolved in water and the BCF is calculated as the ratio of the concentration in the fish to the dissolved concentration in water... However problems can arise with this test method for poorly soluble substances owing to the inability to prepare stable and measurable concentrations in the exposure medium.”



### Bioaccumulation



■ Contaminant levels



■ Contaminant levels

### Biomagnification

Image Source: Scripts Julie van der Hoop

# ANALYTICAL SUPPORT

## Procedure for preparation of spiked fish food

- non-polar, non-volatile, sparsely soluble compounds
- fish test can be performed via dietary test
- fish pellets need to be accurately prepared, homogeneous and stable for a specific time frame



- Trial pellets
  - Sample preparation
    - approximately 0.500 g of the trial pellets were accurately weighed into glass vessels
    - samples were extracted with 20 mL dichloromethane
    - shaking time was 30 seconds
    - extract was 10-times diluted with dichloromethane and analysed



# CHOICE OF ANALYTICAL TECHNIQUE

## Method development

### GC ?

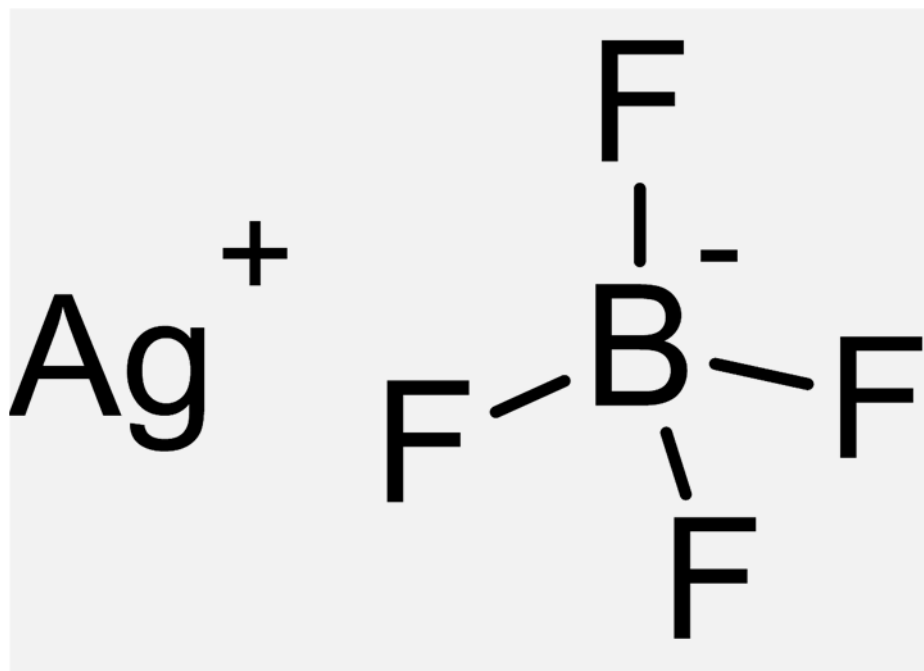
- hydrocarbon-sulphur compounds
- Volatile (<0.001 Pa at 20°C) - no

### UPLC/MS/MS

- non-volatile compounds
- fast
- more suitable for water samples (if needed)
- more sensitive if possible to ionize
- less sample preparation
- **hard to ionize – MS?**

# IONIZE IT - JUST ADD DOPING SALT

Silver tetrafluoroborate



- Lewis acid
- $\text{Ag}^+$  complexes and induces characteristic fragmentation
- doublets in the mass spectrum, because the natural isotopic abundance of silver is 52:48



# ANALYTICAL METHOD

## Analytical conditions

Instrument	Acquity UPLC system (Waters, Milford, MA, USA)
Detector	Xevo TQ-S mass spectrometer (Waters)
Column	Acquity UPLC BEH C8, 100 mm × 2.1 mm i.d., dp = 1.7 µm (Waters)
Column temperature	40°C ± 1°C
Injection volume	2 µL
Mobile phase	Acetonitrile
Post column addition	0.2 mL/min 1 µM Silver tetrafluoroborate in acetonitrile
Flow	0.5 mL/min
MS detection	
Ionisation source	ESI <sup>+</sup>
Cone voltage	50 V
Collision energy	22 eV



# ANALYTICAL METHOD

## Performance of the study

- **Specificity**
  - the blank chromatogram showed no response for the test item or a response of < 30% of the limit of quantification
- **Calibration curve**
  - the response of the calibration solutions was correlated with concentration using regression analysis with a  $1/\text{concentration}^2$  weighting factor
    - coefficient of correlation (r) of > 0.99
    - back calculated accuracies of the calibration solutions in the range 85-115%
- **Accuracy and repeatability**
  - mean accuracy of the quality control samples in the range 70-110%
  - the coefficient of variation  $\leq 20\%$ .
- **Limit of quantification**
  - the lowest concentration level required in the study at which an accuracy in the range 70-110% and a repeatability of  $\leq 20\%$
- **Stability of the analytical system and end solutions**
  - calibration solutions injected throughout the validation sequence including the beginning and end
    - the coefficient of variation on the responses of the solutions  $\leq 20\%$
- **Stability of stock solutions**
  - coefficient of variation on the response factors of the calibration solutions prepared with fresh and stored stock solutions for at least 12 hours  $\leq 10\%$ .

# METHOD VALIDATION

## Results

### Statistical parameters of the calibration curve

<b>Slope</b>	$3.30 \times 10^2$
<b>Intercept</b>	$- 3.76 \times 10^1$
<b>Weighting factor</b>	$1/\text{concentration}^2$
<b>r</b>	0.996

### Stability of the analytical system and end solutions

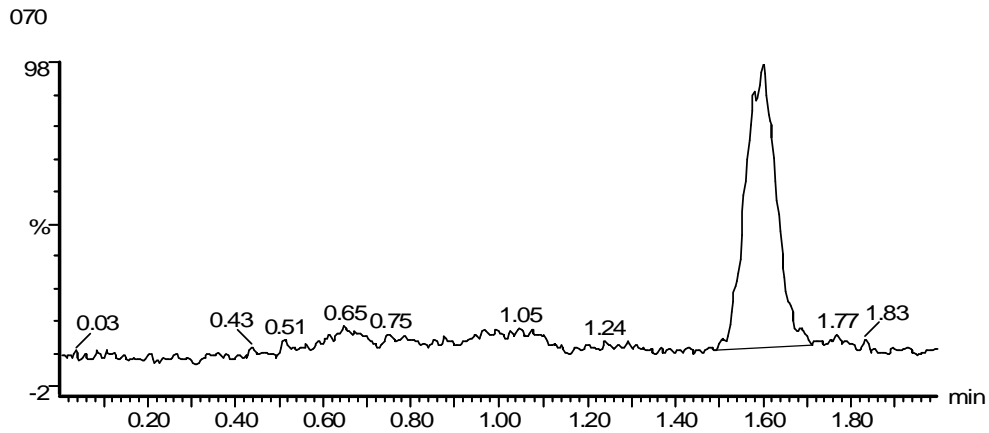
Nominal concentration [µg/L]	Elapsed time [hours]	Coefficient of variation [%]
4.00	1.00	7.8 (n=4)
8.00	1.00	9.3 (n=4)
100	0.85	2.0 (n=4)

### Accuracy and repeatability

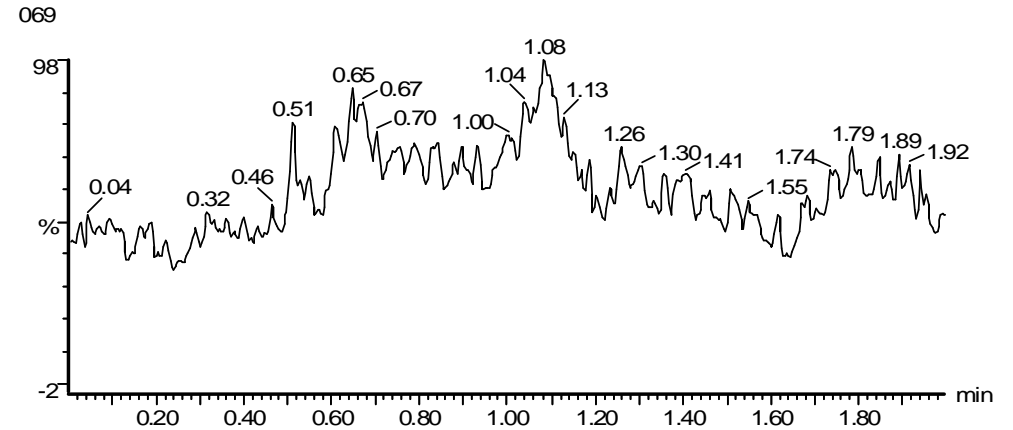
Target	Concentration [µg/g fish food]		Accuracy [%]		Coefficient of variation [%]
	Nominal	Analysed	Individual	Mean	
2	1.99	1.91	96	98	2.3
	1.98	2.00	101		
	1.98	1.90	96		
	2.01	1.98	99		
	2.01	1.93	96		
100	100	109	97	97	7.3
	100	96.4	96		
	100	100	100		
	100	92.2	92		
	100	90.2	90		

# METHOD VALIDATION

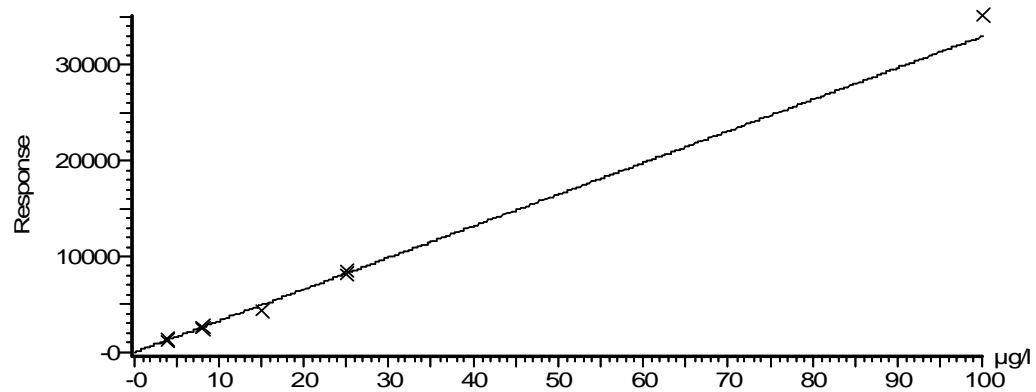
## Results



UPLC-MS/MS chromatogram of a 2 µg/g QC sample



UPLC-MS/MS chromatogram of the blank QC sample



Regression line: response of the test item as a function of concentration

# ACCURACY, HOMOGENEITY AND STABILITY OF PELLETS

## Performance of the study

- **Trial pellets**
  - preparation at a concentration of 5 µg/g
    - dichloromethane was spiked with test item solution and homogenized
    - 50 g pellets were added to get target concentration of 5µg/g
    - dichloromethane was evaporated at 30°C using a rotary evaporator
    - trial pellets were dried overnight at room temperature
- **Acceptance criteria**
  - Accuracy of preparation
    - the mean accuracy was in the range 70-110% of the target concentration
  - Homogeneity
    - if the coefficient of variation was ≤ 15%
  - Stability
    - relative difference between the stored and non-stored samples was maximally 15%

# ACCURACY, HOMOGENEITY AND STABILITY OF PELLETS

## Results

### Accuracy and homogeneity test

Concentration [µg/g fish food]		Accuracy [%]		Homogeneity (coefficient of variation) [%]
Target	Analysed	Individual	Mean	
5	4.59	92	92	2.4
	4.59	92		
	4.66	93		
	4.65	93		
	4.39	88		

### Stability test in the refrigerator for 17 days

Concentration [µg/g fish food]		Accuracy [%]		Relative difference [%]
Target	Analysed	Individual	Mean	
5	4.42	88	89	-2.6
	4.49	90		

## TAKE HOME MESSAGE

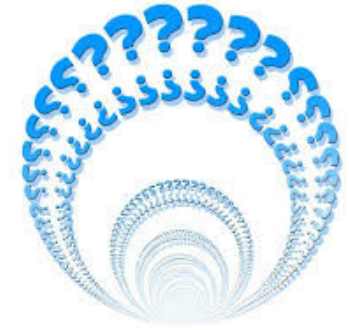
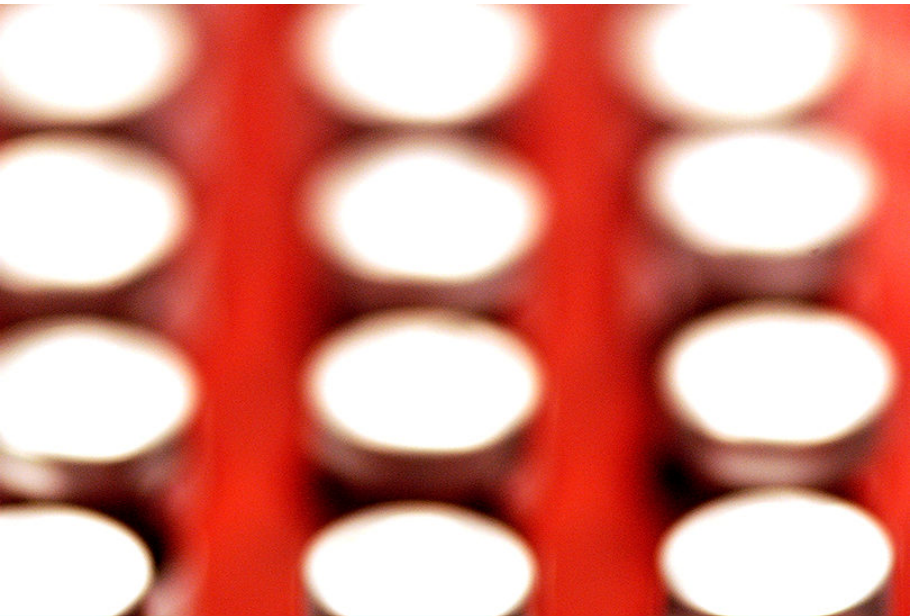
**Method Development**

**Method Validation**

**Preparation of Fish Pellets**

**Analysis of Fish Pellets**

**Evaluation of Data**



# CONTACT US

**Email:**  
[Jelena.Ciric@crl.com](mailto:Jelena.Ciric@crl.com)

**Website:**  
[www.criver.com](http://www.criver.com)