Società Chimica Italiana
Divisione di Spettrometria di Massa

ARPAC
CAMPANIA

4th MS Envi Day

Napoli

October 1-3, 2018

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The 4th MS Envi Day

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Monday, October 1st, 2018

13:30 – 14:00 Registration and reception

14:00 – 14:30 **Welcome addresses**

14:30 **1st Session**

Chair: ***Donatella Caruso*** *(Università di Milano)*

14:30 – 15:00 **ATTIVITÀ DI ARPAC SULLA "TERRA DEI FUOCHI"**

Marinella Vito

ARPA Campania, Napoli

15:00 – 15:30 **MONITORAGGIO DI MICROINQUINANTI ORGANICI NEL TERRITORIO DELLA "TERRA DEI FUOCHI": METODOLOGIA E RISULTATI**

Luigi Iannibelli

ARPA Campania, Pozzuoli

15:30 – 16:00 **DETERMINATION OF SEMI-VOLATILE COMPOUNDS IN WATER BY AUTOSPME/MULTIEXP/ FAST-GC-MS AND GC-MS/MS. STATE-OF-THE-ART IN THE ARPAE LABORATORY IN BOLOGNA**

Rino Calori, Cecilia Bergamini, Maria Ferrari, Manuela Di Giovanni, Maurizio Falchieri, Barbara Romagnoli, Emanuela Fabbrizi

Arpae Emilia Romagna - Laboratorio Multisito sede Secondaria di Bologna

16:00 – 16:30 **CONSIDERAZIONI E INQUADRAMENTO IN ACCORDO ALLA DIRETTIVA QUADRO 2008/56/CE, SULLA PRESENZA E SULLA DISTRIBUZIONE DI MICROINQUINANTI ORGANICI (PCB E PBDE) IN SEDIMENTI E BIOTA IN TOSCANA**

Valeria Filippi, Federica Bellandi, Michele Mazzetti, Paolo Altemura

ARPA Toscana, Laboratorio AVL, Livorno

16:30 *Guided tour to Palazzo Reale*

18:30 *Welcome cocktail – Grand Caffè Gambrinus*

Tuesday, October 2nd, 2018

9:00 **2nd Session**

Chair: ***Cecilia Bergamini*** *(ARPAE Emilia Romagna, Bologna)*

9:00 – 9:45 **CONTAMINANT SCREENING IN THE AQUATIC ENVIRONMENT USING HIGH RESOLUTION MASS SPECTROMETRY – METHODOLOGIES AND REAL WORLD APPLICATIONS**

Heinz Singer, Sabine Anliker, Michele Stravs, Christoph Ort, Steffen Ruppe, Juliane Hollender

Eawag, Environmental Chemistry, Dübendorf, Switzerland

9:45 – 10:15 **LCMS & GCMS: COMPLEMENTARY TECHNIQUES FOR PESTICIDES ANALYSIS**

Franco Bruno

Shimdzu Italia S.r.l.

10:15 – 10:45 **DETERMINAZIONE DI PFASs IN MATRICI AMBIENTALI CON LA TECNICA LC-MS/MS: CARATTERISTICHE, VANTAGGI E CRITICITÀ**

Alessandro Pellizzaro

Acque del Chiampo SpA, Arzignano (VI)

10:45 – 11:15 *Coffee break*

11.15 **3rd Session**

Chair: ***Valeria Filippi*** *(ARPA Toscana, Livorno)*

11:15 – 11:45 **QUANTITATIVE ANALYSIS AND UNTARGETED SCREENING IN WATER INTENDED TO HUMAN CONSUMPTION BY HIGH RESOLUTION MASS SPECTROMETRY**

Lydia Balest, Pier Paolo Abis

Acquedotto Pugliese S.p.A, Bari

11:45 – 12:15 **MONITORING OF PERFLUOROALKYL SUBSTANCES (PFAs) IN TUSCANY:METHODOLOGY AND RESULTS**

Michele Mazzetti, Valeria Filippi, Paolo Altemura, Guido Spinelli

ARPA Toscana, Laboratorio AVL, Livorno

12:15 – 12:45 **INQUINAMENTO ATMOSFERICO NELLA PROVINCIA DI NAPOLI**

Marco Trifuoggi

Dipartimento di Scienze Chimiche, Università degli Studi di Napoli "Federico II", Napoli

13:00 – 14:30 *Buffet Lunch*

14.30 **4th Session**

Chair: ***Salvatore Di Rosa*** *(ARPA Campania)*

14:30 – 15:15 **LC-MS BASED STRATEGIES FOR THE COMPREHENSIVE ANALYSIS OF MARINE TOXINS IN ENVIRONMENTAL AND FOOD MATRICES**

Carmela Dell'Aversano, Luciana Tartaglione

Department of Pharmacy, School of Medicine and Surgery, University of Napoli Federico II, Napoli (Italy)

15:15 – 15:45 **DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS (PCDDs), POLYCHLORI-NATED DIBENZOFURANS (PCDFs) AND POLYCHLORINATED BIPHENYLS (PCBS) IN HENS EGGS**

Sara Lambiase,[1,2] Filomena Fiorito,[1] Francesco P. Serpe,[1] Pasquale Maglio,[1] Alfredo Scaramuzzo,[1] Marco Trifuoggi,[2] Mauro Esposito [1]

[1] Department of Chemistry - Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (Naples, Italy)
Department of Chemistry - Istituto Zooprofilattico Sperimentale del Mezzogiorno)

15:45 – 16:15 **DETERMINATION OF MERCURY IN SEA WATERS AND BIOTA (FISH & MOLLUSCS) BY AAS & ICP-MS**

Elisa Di Alessandro, Franco Castellani, Rosella Filardi, Romano T. Baino, Carlo Cini

ARPA Toscana, Laboratorio AVL, Livorno

16:15 – 16:45 *Coffee break*

16:45 **5th Session**

16:45 – 17:15 **MONITORAGGIO DEL TRIBUTIL STAGNO CLORURO IN TOSCANA: METODOLOGIA E RISULTATI**

Federica Bellandi, Valeria Filippi, Michele Mazzetti, Paolo Altemura

ARPA Toscana, Laboratorio AVL, Livorno

17:15 – 17:30 **POLYCYCLIC AROMATIC HYDROCARBONS AND RELATIVE CORRELATION WITH PM LEVELS AND INORGANIC FRACTION IN APPLE FRUITS: A PRELIMINARY STUDY IN MOLISE REGION**

Ivan Notardonato, Cristina Di Fiore, Pasquale Avino

Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso

17:30 *End of session*

20:30 *Social Dinner*

Wednesday, October 3rd, 2018

9:20 **6th Session**

Chair: ***Gianluca Giorgi*** *(Università di Siena)*

9:20 – 9:50 **A MODIFIED EPA METHOD FOR SIMULTANEOUS DETERMINATION OF CHLORINATED ORGANIC POLLUTANTS IN SOIL AND SEDIMENTS BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY**

Francesco Cardellicchio,[1] Giuseppe Mascolo,[2] Francesco Palmisano [1]

[1] Department of Chemistry, University of Bari, Bari
[2] CNR- IRSA, Bari

9:50 – 10:20 **DETERMINATION OF ESTROGENIC ENDOCRINE DISRUPTORS AT pg L^{-1} LEVELS IN WATER SAMPLES, ACCORDING TO DECISION 2015/495/EU**

Maddalena Busetto, Luisa Colzani, Laura Clerici, Pierluisa Dellavedova

ARPA Lombardia, U.O. Laboratorio di Milano

10:20 – 10:50 **HPLC-ESI(+)-MS/MS METHOD FOR SCREENING AND TRACE LEVEL DETERMINATION OF PHARMACEUTICALS IN AQUEOUS ENVIRONMENTAL SAMPLES**

Raffaella Pascale, Maria Cristina Lafiosca, Donatella Caniani, Salvatore Masi, Ignazio M. Mancini, Alberto Onzo, Donatella Coviello, Laura Scrano, Sabino A. Bufo, Giuliana Bianco

Università della Basilicata, Potenza

10:50 – 11:10 *Coffee break*

11.10 **7th Session**

11:10 – 11:50 **SPECIAZIONE DEGLI IDROCARBURI PESANTI SECONDO IL METODO MA.D.E.P. IN GC-FID/MS – CARATTERIZZAZIONE DEI SUOLI DI BAGNOLI**

Salvatore Di Rosa

ARPA Campania, Napoli

11:50 – 12:10 **GLYPHOSATE AND AMPA: METHODOLOGY & RESULTS**

Michele Mazzetti

ARPA Toscana, Laboratorio AVL, Livorno

12:10 – 12:40 *Round table*

12:40 – 12:55 *Concluding remarks & Arrivederci!!*

ABSTRACTS

DETERMINATION OF SEMI-VOLATILE COMPOUNDS IN WATER BY AUTOSPME/MULTIEXP/FAST-GCMS-GCMSMS STATE-OF-THE-ART IN THE ARPAE LABORATORY IN BOLOGNA

Rino Calori, Cecilia Bergamini, Maria Ferrari, Manuela Di Giovanni, Maurizio Falchieri, Barbara Romagnoli, Emanuela Fabbrizi

Arpae Emilia Romagna - Laboratorio Multisito sede Secondaria di Bologna

The laboratories that deal with environmental analysis have encountered in recent years a number of operational problems: a greater number of samples to be analyzed, the need for fast, accurate answers, cost containment limitations and, and critically, in lowering the legal limits of the substances to be analyzed.
The introduction of Fast GC combined with new multi-extraction techniques, combined with new instruments, allowed to improve and in many cases to solve many of these problems.
The Fast GC maintains the same separation of the traditional type gas-chromatography, despite the decrease in analysis time ensures linear responses for quantitative analysis and, in the case of mass spectrometers, spectra comparable with the public libraries. The necessary instrumental analysis times can be reduced by a factor of 5, and in some cases even higher.
For the analysis of environmental samples[1] Fast GC coupled to 'autoSPME (Automated Solid Phase Micro Extraction) ensures precision and accuracy while maintaining the very short analysis times.
SPME is based on the equilibrium that is established between the phase deposited on the fiber and the matrix in which the substance to be analyzed is present, once the equilibrium is reached, dependent on all operating conditions, the quantity of substance present on the fiber, proportional to the initial concentration in the matrix, remains constant, despite its capacity to retain larger quantities.
A limit of our automatic SPME system is given by the small amount of matrix, in our case, water, necessary for analysis, at most twenty milliliters.
To increase the amount of sample, and consequently the quantity of sampled analyte, it is possible to introduce the fiber sequentially into several vials: the SPME theory confirms the increase in the quantity of the retained substance and consequently an increase in the analytical response.
This theory has been confirmed by laboratory tests on semi-volatile substances such as PAH, PCB'S and phenols.
Analytical methods have been studied and developed for the detection of these semi-volatile compounds in water, using these separation and extraction techniques and, in the case of PCB's and phenols, not by dipping the SPME fiber in the sample but by sampling the head space, commonly technique used only for the volatile substances.
This innovation allows to also analyze any type of sample because the fiber is not in

contact with the matrix but only with the analytes present in the headspace.
All variables that influence the in the SPME analysis have been studied, such as the type of fiber, the time and the temperature of the sample.
Some methods have been validated and are periodically checked using "proficiency test".

1. J. Pawliszyn , Handbook of Solid Phase Microextraction. Chemical Industry Press (2009)

CONSIDERAZIONI E INQUADRAMENTO IN ACCORDO ALLA DIRETTIVA QUADRO 2008/56/CE, SULLA PRESENZA E SULLA DISTRIBUZIONE DI MICROINQUINANTI ORGANICI (PCB E PBDE) IN SEDIMENTI E BIOTA IN TOSCANA

Valeria Filippi, Federica Bellandi, Michele Mazzetti, Paolo Altemura

ARPAT - Agenzia Regionale per la Protezione Ambientale della Toscana – Laboratorio AVL, via G. Marradi 114 -116, Livorno

Summary: *Applicazione della direttiva quadro 2008/56/CE e del D.Lgs. 172/15. Metodo multiparametrico di determinazione di PCB e PBDE: risultati e correlazioni tra i diversi inquinanti.*

Keywords: *MSFD, direttiva 2008/56/CE, D.Lgs. 172/15, Biota, PCB, PBDE*

La Direttiva quadro sulla strategia per l'ambiente marino **2008/56/CE** (**MSFD, Marine Strategy Framework Directive**), entrata in vigore nel luglio del 2008, rappresenta un importante e innovativo strumento per la protezione dei nostri mari poiché costituisce il primo contesto normativo, vincolante per gli Stati Membri (SM) della Unione Europea, che considera l'ambiente marino in un'ottica sistemica. La MSFD richiede agli SM di sviluppare e attuare delle "strategie marine" con lo scopo di proteggere e preservare l'ambiente marino, prevenirne il degrado o, dove possibile, procedere al ripristino degli ecosistemi marini nelle aree in cui abbiano subito impatti, ciò al fine di conseguire o mantenere un buono stato ambientale (GES, Good Environmental Status) di mari e oceani entro il 2020.
Per consentire agli Stati membri di raggiungere gli obiettivi prefissati, la direttiva ha sviluppato 11 descrittori qualitativi per la determinazione del buono stato ambientale degli ecosistemi. Tra i diversi descrittori (di tipo biologico, geologico, fisico), quelli di nostro interesse sono:
Descrittore 8 - Le concentrazioni dei **contaminanti** presentano livelli che non danno origine a effetti inquinanti.
Descrittore 9 – I **contaminanti presenti nei pesci** e in altri frutti di mare destinati al consumo umano non eccedono i livelli stabiliti dalla legislazione comunitaria o da altre norme pertinenti.
L'Italia ha recepito la MSFD con il **D.lgs. 190/2010**.
Già dal 2015 Arpat effettuava monitoraggio di sostanze chimiche in biota (mitili) quali PCB ed alcuni pesticidi.
Nel 2017 si è proceduto ad estendere la ricerca di tali sostanze pericolose anche nel pesce, sia di acque fluviali che di transizione, come previsto dal **D.Lgs 172/15**. Le attività di campionamento ed analisi sono state eseguite in accordo alle linee guida ISPRA "linee guida per il monitoraggio delle sostanze pericolose (secondo il D.Lgs 172/15)". La ricerca di sostanze pericolose nel biota contribuisce alla classificazione

dello stato chimico in acque fluviali e di transizione. Applicando i criteri e il confronto con gli standard di qualità ambientale del D.Lgs 172/15, tutti i campioni di biota si sono collocati ad un livello “non buono” per superamento di alcuni parametri (con frequenza maggiore quelli corrispondenti al mercurio e al **difeniletere bromurato (PBDE)**).

Le specie ittiche, pescate per la ricerca di sostanze pericolose nei loro tessuti, sono state Liza Ramada e Mugil Cephalus conosciuti come Cefalo Calamita e Cefalo Comune.

Sfruttando l’esperienza acquisita con l’analisi di PCB nei mitili, si è proceduto quindi alla messa a punto di un metodo **multiparametrico** per l’estrazione e purificazione di DIOSSINE e FURANI, PCB e PBDE.

Il campione da sottoporre ad analisi è stato addizionato di una idonea quantità di materiale di riferimento isotopicamente arricchito (Diossine, Furani, PCB e PBDE marcati al ^{13}C) ed estratto mediante l'uso dei QuEChERS (acronimo di Quick, Easy, Cheap, Effective, Rugged and Safe). L’ARPA Toscana è stata tra le prime agenzie ad adottare tale metodo estrattivo su matrici non alimentari (terreni, sedimenti) e ad estenderlo a contaminanti organici non consueti per tale metodo (diossine, furani, PCB, PBDE). La purificazione è stata effettuata con una colonna multistrato (seguendo il metodo EPA 1614 specifico per PBDE) in automatico utilizzando lo strumento Gilson GX271 oppurtunamente configurato per la purificazione. Come tecniche strumentali sono state utilizzate tecniche in spettrometria di massa GC/HRMS Magnetica (per Diossine e PCB), GC/MS/MS (PBDE).

I risultati ottenuti hanno contribuito a stabilire un quadro NON BUONO circa lo stato chimico delle acque.

Inoltre, dall’analisi dei dati ottenuti, di notevole interesse è risultata: la correlazione tra la concentrazione degli inquinanti nelle varie matrici:

1. la correlazione, per punto di campionamento, tra le concentrazioni di PCB in sedimenti e biota
2. la distribuzione, per punto di campionamento, di PCB e PBDE in biota.

CONTAMINANT SCREENING IN THE AQUATIC ENVIRONMENT USING HIGH RESOLUTION MASS SPECTROMETRY – METHODOLOGIES AND REAL WORLD APPLICATIONS

Heinz Singer, Sabine Anliker, Michele Stravs, Christoph Ort, Steffen Ruppe, Juliane Hollender

Eawag – Swiss Federal Institute of Aquatic Science and Technology, Duebendorf, Switzerland

Pharmaceuticals, pesticides, and industrial chemicals can enter the aquatic environment via different pathways after their use in households, their application on agricultural fields, or their industrial production. In the environment those contaminants can pose potential (eco-) toxicological risk to humans and aquatic organisms even at the nanogram per liter level. Furthermore, abiotic and biotic transformation processes can form a multitude of transformation products in the water cycle. To study the occurrence and fate of parent compounds and transformation products, selective and sensitive methods, suitable for the detection of compounds with a wide range of physico-chemical properties, are needed. For this purpose, liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) is nowadays the method of choice due to its capability to screen simultaneously for target, suspect and non-target compounds. Sample enrichment (i.e. by solid phase extraction, direct large volume injection, vacuum-assisted evaporation) is often required and a key factor for a sensitive detection. Adequate data processing workflows comprise data pre-processing steps (i.e. peak detection, blank subtraction, replicates filtering, isotope and adduct grouping), prioritization of the pre-processed data (i.e. by mass defect, time or spatial trends, clustering, eco-toxicological effects), and identification of prioritized components (i.e. by library searches, suspect searches with online databases and meta information, in-silico predictions). [1]

Hence, the whole non-target workflow involves many different steps from sample preparation to compound identification. For a successful identification of a suspect or non-target compound a proper adjustment of each single workflow step is essential. While for metabolomics or proteomic experiments sophisticated LC-HRMS measurement setups and data processing strategies became established, environmental applications pose challenges as highly dynamic concentration changes in matrix-rich samples such as wastewater need to be monitored under cost and time restrictions.

To prove that successful target, suspect and non-target analysis is possible with such constraints under real world condition, the daily LC-HRMS screening at the international Rhine monitoring station will be showcased. Based on selected case examples, the performance of automated on-time data processing with time-trend

analysis using the open source software enviMass will be demonstrated for the Rhine monitoring. In 2014, a cumulative load of 100 tons of household chemicals were detected by LC-HRMS screening in the river Rhine at Basel. These chemicals were continuously released from municipal waste water treatment plants. Additionally ten major industrial spill events of previously undetected compounds were identified in 2014, corresponding to over 25 tons of chemical load in the Rhine at Basel. Subsequently, upstream investigations in the river catchment revealed the responsible polluters and mitigation measures have since been installed. [2, 3]
However, the acquisition of long-time profiles with high temporal resolution implies extraordinary efforts for sampling and transport. To overcome this limitation we are currently developing a transportable mass spectrometry platform placed in a trailer. First tests within the MS2FIELD project with an optimized Orbitrap system using online direct injection of continuously pre-filtered water showed good stability and interesting high temporally resolved results over a period of one week unattended measurements in waste water influent. [4]

1. J. Hollender, E. Schymanski, H. Singer, L. Ferguson, Environ. Sci. Technol., **51**, 11505-11512 (2017)
2. M. Ruff, M. Mueller, M. Loos, H. Singer, Water Research, **87**, 11505-11512 (2017)
3. S. Ruppe, D. Griesshaber, I. Langlois, H. Singer, J. Mazacek, Chimia, **72**, 547 (2018)
4. https://www.eawag.ch/en/department/sww/projects/ms2field/

LC-MS & GC-MS: COMPLEMENTARY TECHNIQUES FOR PESTICIDES ANALYSIS

Franco Bruno

Shimdzu Italia S.r.l.

Summary: *LC-MS/MS is a powerful technique for the quantifications and identification of small molecules at trace level: the problem of false positive/negative reporting could affect its reliability. To overcome this limitation we reports a novel acquisition mode, able to produce high quality and reliable spectra. In GCMS Shimadzu offers databases with a large number of compounds for pesticides and pollutants including MRM, Collision Energy, LRI for specific capillary columns stationary phases.*

Keywords: MRM Spectrum mode*, Collision Energy, Triple Quadrupole*

In LCMS/MS, to help reduce the incidence of false positive and false negative reporting in pesticide residue monitoring routine multiple-reaction monitoring (MRM) methods have been enhanced to monitor a higher number of fragment ion transitions to increase specificity and reporting confidence.
In this workflow, typically 6-10 fragment ion transitions were monitored for each target pesticide as opposed to a conventional approach using 2-3 fragment ions. By acquiring a high number of fragment ion transitions, each target pesticide had a corresponding fragmentation spectra which could be used in routine library searching and compound verification using reference library match scores. This 'MRM Spectrum mode' can be applied to quantify and identify about 200 pesticides using thousands MRM transitions without compromising limits of detection, linearity or repeatability using a high speed data acquisition triple quadrupole MS/MS. To complete the full panel of regulated pesticides, GCMSMS became the complementary technique to analyze compounds for which LCMS sources show low ionization.
In GCMS Shimadzu offers some databases with a large number of compounds for pesticides and pollutants. Each of them includes MRM, Collision Energy, LRI for specific capillary columns stationary phases, etc. for any compound.
The biggest advantages of these database allow to prepare a complete GCMS/MS method simply by installing the capillary column and inject a mix of hydrocarbons. The SW automatically calculates the LRI and creates a MRM acquisition method for hundreds of selected analytes. In the same way it allows to correct the MRM table and recalculate the retention times in case of maintenance or replacement of the GC column

QUANTITATIVE ANALYSIS AND UNTARGETED SCREENING IN WATER INTENDED TO HUMAN CONSUMPTION BY HIGH RESOLUTION MASS SPECTROMETRY

Lydia Balest, Pier Paolo Abis

Acquedotto Pugliese SpA

Summary: *A monitoring plan of water bodies was conducted by targeted and untargeted approach: a number of unknown compounds were identified focusing on a limited number of prioritized peaks ranged from a preliminary PCA study. It was possible to identify a list of priority compounds in water bodies of interest.*

Keywords: *screening in water, ultratrace detection, non target screening.*

Introduction

In order to empower the surveillance level in surface water intended for human consumption, a monitoring plan of water bodies was conducted by both targeted untargeted approach. Analytical methods for some new emerging pollutants such as pesticides, pharmaceutical compounds, endocrine disrupters and suspect cyanotoxins were investigated by high resolution mass spectrometry (LC-QqTOFMS) for quantitative analysis. In particular, because of the natural occurrence of seasonal algal bloom in water bodies, monitoring cyanotoxyns as a byproduct of blue alge is one of the main focus for monitoring plans of water managements.This work intended to assist public drinking water managements for evaluating their source waters for vulnerability to contaminations. For this pourpose identification of organic pollutants in aquatic ecosystem is one of the most essential concerns with respects to human health and aquatic life. It is important to note that, usually, most chemical screening studies are focused on known components (i.e. pharmaceuticals, pesticides, POPs, natural occurring molecules, etc.) and based on commercial library database software, while many peaks in LC-MS chromatograms are often unknown and ignored in chemical monitoring. monitoring

Experimental

An HPLC system Nexera-X2-30AD (Shimadzu), equipped with a CBM-20A module was coupled to a X500R QTOF mass spectrometer (Sciex) equipped by an electrospray ionization (ESI) source and a HTS Pal autosampler (CTC Analytics). Untargeted analysis were conducted using Luna C18(2)-HST column, 100 Å, 2.5µm, 100 x 3mm (Phenomenex). Mobile phase A was a 0.1% (v/v) solution of formic acid in water, mobile phase B 0.1% (v/v) solution of formic acid in metanol. MS analysis was performed using positive electrospray ionization mode both in TOF/MS and IDA experiment mode. Post processing of accurate mass measurements were done

by Sciex OS 1.2 software and HR-MS All in one library. Multiview 1.3.1 software was used for PCA analysis.

Results

During surface waters and ground water surveillance monitoring, multi residual quantitative methods developed by HPLC/HR-MS were applied for quantification of ibuprofen, diuron, 2,4-chloro-2-methylphenoxy acetic acid (MCPA), 17alfaethyinilestradiol (EE2), Bisphenol A, Nonyl-phenol, glifosate, glufosinate, (a-amino- 3-hydroxy-5-methyl-isoxazolepropionic acid (AMPA) and polyfluoroalkylsubstances (PFAS) at sub ug/L levels.

In particular, an HPLC/HR-MS quantification method for eleven different congeners of MCs and dm-MCs, nodularin, cylindrospermopsin, anatoxin and saxitoxins and domoic acid in raw and treated surface water was implemented. At the same time an untarget screening was achieved for different congeners of cyanotoxins and/or their byproducts.

Moreover a number of unknown compounds were identified focusing on a limited number of prioritized peaks ranged from a preliminary PCA study. It was possible to identify a list of priority compounds in water bodies and groundwater of interest. The determination of the molecular formula for unknown peaks was based on accurate masses, while the most likely structure among hundreds or thousands of possible candidates retrieved from a database search and software- based predictions.

Such approaching model, based on multivariate analysis of HPLC/HR-MS data allowed to obtain an overview to map out for m the state of water quality, relating to the content of organic natural substances or pollutants, over different seasons. In addition, such multivariate model highlight similarity or differences among water samples.

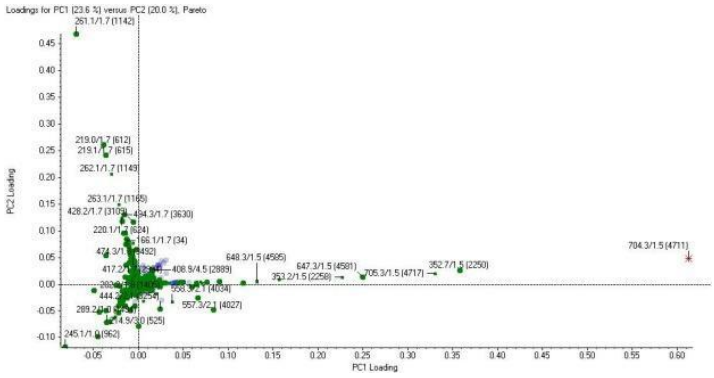

Fig. 1 . Loading plot obtained from PCA analysis performed on HPLC/HR-MS

Conclusions

While target screening depends on the use of reference standards for identification and quantification, suspects screening is based on known compound masses. Because of the lack of reference materials for most microcystins congeners, an evaluation of presence of many congeners and/or their environmental metabolite in water body was achieved by accurate mass search.

In addition, nontarget screening strategy has been developed to detect and identify unknown compounds without any prior knowledge solely from the analytical data. For this purpose, In a first step, a list of all peaks containing accurate m/z, retention time, and suggested formula from HPLC-HRMS data has been generated. The quality of results depends on a good adjustment of the software settings to the analytical data and requires careful optimization of instrumental conditions. Moreover, co-eluting peaks originated from the same compound such as isotopologues, adducts formed in the ion source or in-source fragments have been identified. In a second step, each molecular formula candidate compound lists have been retrieved from ChemSpider, a chemical compound databases, in order to reduce the large candidate lists obtained, evaluating MS/MS fragmentation prediction. Nontarget screening by accurate mass measurements may be a powerful tool to investigate their occurrence in different water bodies and to profile a kind of "finger print" of different water samples.

References

1. Smital, T., Terzic, S., Lonear, J., Senta, I., Žaja, R., Popovic, M., Mikac, I., Tollefsen, K.-E., Thomas, K.V., Ahel, M., 2013. Prioritisation of organic contaminants in a river basin using chemical analyses and bioassays. Environmental Science and Pollution Research 20, 1384-1395.
2. Terzic, S., Ahel, M., 2011. Nontarget analysis of polar contaminants in freshwater sediments influenced by pharmaceutical industry using ultra-high-pressure liquid chromatography– quadrupole time-of-flight mass spectrometry. Environmental Pollution 159, 557-566.

MONITORING OF PERFLUOROALKYL SUBSTANCES (PFAS) IN TUSCANY: METHODOLOGY AND RESULTS

Michele Mazzetti, Valeria Filippi, Paolo Altemura, Guido Spinelli

ARPAT - Agenzia Regionale per la Protezione Ambientale della Toscana

Summary: *A HPLC-HRMS method for analysis of perfluoroalkyl substances was developed and applied in monitoring activity of environment of Tuscany region.*

Keywords: *PFAS, HPLC-HRMS, biota*

Since the middle of the last century, fluorinated surfactants (perfluoroalkylsulfonic derivatives and subsequently perfluoroalkyl acids) have been commercially available.
The charateristic physical-chemical properties, such as the lowering of surface tension in aqueous systems and the high chemical and thermal stability, were the basis of the commercial success of these substances and of their enormous use in a wide range of industrial processes.
The widespread use, the disposal of these compounds and the low efficiency of environmental degradation processes, has led to the widespread presence of PFAS in every environmental sector with a particular preference for the water sector.
The two most commonly used PFASs found in the environment are perfluoro-octanulfonic acid (PFOS) and perfluorooctanoic acid (PFOA).
These substances are considered persistent, bioaccumulative and toxic (PBT / vPvB) according to Annex VI of the EC Regulation 1272/2008.
The European Commission has limited and regulated the use of PFOS in the Union and such substances are included in the list of priority substances, setting, in the Directive 2013/39.
In this legislative act an environmental quality standard (EQS) is set for superficial waters, the EQS is expressed both as an annual mean concentration (EQS-AA) and as a maximum allowable concentration (EQS-MAC).
Moreover, with the purpose of classifying the ecological status, an EQS-MAC, regarding to groundwaters, is stated for further five substances, four of which belong to the perfluorocarboxylic acid category and one to that of perfluorosulfonic acids.
The limits for PFAs for surface waters are established in Legislative Decree No. 172 of 13/10/15 (that will come into force in December 2018), while those for groundwaters are set in MATTM Decree of 6 July 2016.
Considering that the monitoring activity is mandatory at European level, in 2016 ARPA Toscana developed a method of analysis of these compounds based on on-line solid phase extraction (SPE) and on detection and quantification through high performance chromatography interfaced with a high resolution mass selection spectrometer system (HPLC-HRMS) based on Orbitrap technology.
The developed analytical method allows the determinationof perfluorosulfonic acids

at the level of 0.5 ng / L (0.0005 µg / L) and of perfluorocarboxylic acids at the level of 2 ng / L (0.002 µg / L) ,in surface waters and groundwater.
This method has been validated through a collaboration with IRSA-CNR (duplicate analysis of samples collected in Tuscany and analyzed by IRSA during 2013) and through participation in interlaboratory circuits (AGLAE).
During last year 13 samples of water from Arno River and additional 29 samples from further 11 surface water bodies were analyzed for PFAS.
Regarding groundwater, PFAS determinations were carried out in 40 wells, in the following aquifers: Sieve, Pitigliano volcanites, coastal aquifer between Cecina and San Vincenzo, coastal between Fine and Cecina, the Cornia plain, Cerbaie and Bientina deep water, Valdarno lower and coastal plain of Pisa, Val di Nievole Fucecchio area.

Sample Types		***Matrix***	***Number of Points***	***Number of Samples***	***Number of Determinations***	***Number of Determinations > LOQ***
Surface Waters	Rivers and Lakes	Water	14	42	252	169
		Biota	15	16	16	16
	Transitional Marine and Coastal	Water	1	5	30	21
		Biota	18	19	19	18
Ground Water		Water	40	74	444	46
G.W. and S.W. for drinking production Production		Water	4	4	19	6
Total			92	160	780	276

The evaluation of the obtained analytical results highlights 276 determinations with a value higher than the analytical quantification limit (LOQ), with values also significantly close to the SQA-MA for perfluorooctanoic acid (PFOA) and perfluorbutanulphonic acid (PFBS); the limit for perfluorooctansulphonic acid (PFOS) is exceeded, on 12 stations of the 14 considered.
Related to a geographic analysis of the results, it is possible to identify a localization of the most critical situations in the Arno river (middle valley section) and of some of its tributaries (Bisenzio, Elsa Usciana) some situations of less concern can be found in some water bodies of the coast.
Some the underground aquifer most affected from presence of PFAS are those in Pistoia district.
During 2017 35 PFOS determinations were carried out on samples of freshwater fish fauna (*Barbus plebejus, Salmo truta* ecc.) and sea fish fauna (*Leuciscus cephalus, Liza aurada, Liza ramada* etc.) in compliance with Legislative Decree. 172 of 13/10/2015 and the Marine Strategy project (European Directive 2008/56 / EC and Legislative Decree 190/2010),
The ARPAT analytical method for biota implied a modified Quechers extraction, the

purification of extracts by extraction in solid phase (SPE) on weak anionic exchange columns (WAX) and the detection and quantification by high performance chromatography interfaced with a spectrometric system with selection of High resolution mass (HPLC-HRMS) based on Orbitrap technology: this allows confirmation and determination of the analyte in question (PFOS) with a LOQ of 0.5 µg / kg.
The concentantion of PFOS found in all samples was higher than LOQ but less than biota EQS (9.1 µg / kg) stated in Dlgs. 172 of 13/10/2015 .
Situations close to the EQS were found on specimens of *Liza ramada* captured near the estuarine of the Arno and with a low concentration on the Livorno coast.
On these samples, using retrospective analysis provided by the Orbitrap system, it was possible to identify the typical ion traces of perfluorocansulfonamide (PFOSA), a substance used in various types of water-repellent formulations and perfluoro-chansulphonate precursors.

LC-MS BASED STRATEGIES FOR THE COMPREHENSIVE ANALYSIS OF MARINE TOXINS IN ENVIRONMENTAL AND FOOD MATRICES

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Microalgae are vitally important to marine ecosystems and most of the microalgal species are not harmful. However, an important number of species produces potent, heat-stable toxins. Concomitantly to harmful algal blooms (HAB), these toxins can be transferred to humans mainly through the food web but also through other exposure routes, such as aerosol and/or skin contact, or even cause mortality of aquatic organisms. These events result in adverse effects and sanitary problems, as well as in significant economic losses related to aquaculture, fishery and tourism sectors.

Efficient analytical strategies are thus required to detect toxins in the environment and in food supply to the final aim of protecting human health and guaranteeing seafood safety and quality. Selection of the appropriate instrumental technique may be challenging because marine toxins are a heterogeneous group of structurally complex compounds, usually contained at sub-mg levels in complex matrices in the form of a wide array of different congeners. In addition, certified reference material of individual toxins is in some cases unavailable, which hampers full validation of analytical methods. The combination of liquid chromatography with mass spectrometry (LC-MS) is pointed by official organizations, such as the European Food Safety Authority (EFSA), as the most promising instrumental technique for the monitoring of marine toxins in the environment and in seafood.

Several MS-based approaches employing either tandem MS or high resolution MS^n have been developed so far for the detection of both the regulated and the emerging toxins, validated, and eventually used as an effective alternative to animal based methods in official monitoring of marine toxins in seafood. They allowed to disclose the presence of known and unknown toxins in the Mediterranean area and even to structurally characterize new congeners based on the interpretation of their fragmentation patterns [1].

An overview of the different experimental strategies used to discover marine toxins, determine toxin profile and content of algal and mussel samples, and elucidate the structure of new low-, mid- and high-MW congeners will be presented. Although LC tandem MS proved useful to face most of the HAB-related outbreaks occurred so far due its high sensitivity, selectivity and reproducibility, in some cases it lacked in providing a comprehensive overview of the toxin profile of a real sample. That's where high resolution MS^n played a key role, proving to be the most desirable approach to avoid underestimation of sample toxicity.

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DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS (PCDDs), POLYCHLORINATED DIBENZOFURANS (PCDFs) AND POLYCHLORINATED BIPHENYLS (PCBs) IN HENS EGGS

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Summary: *the aim of the work was to validate a method for the analysis of dioxins and DL-PCBs in hens eggs for the use in routinely activity. Levels of dioxins and DL-PCBs found in eggs from free-range hens were compared with eggs from conventional production systems, showing higher levels.*

Introduction

Dioxins (PCDDs and PCDFs) and PCBs are persistent environmental pollutants, associated with several human health effects mainly linked to endocrine interference. The aim of this work was to validate a method for the quantitative analysis of 7 PCDDs, 10 PCDFs and 12 dioxin-like PCBs (DL-PCBs) in hens eggs which are considered one of the most common and cheap food for human consumption giving in the early years several exceedings of maximum permitted limits set by Commission Regulation EU 1259/2011 [1].

Experimental

Eggs samples from different conventional production systems were used for the validation study.

The method was developed in accordance with US EPA method 1613 revision B and 1668 revision C and employed the isotope dilution technique. Pre-treatment of samples consisted of freeze-drying and extraction using an Accelerated Solvent Extraction (ASE, Thermo Fisher Scientific) system, clean up using first a multilayer acid column and then a Power Prep system (FMS).

The instrumental analysis was conducted using a high-resolution gas chromatograph coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific) operating at a resolution of 10000.

A total of 56 egg samples from free-range hens were analyzed for the presence of dioxins and DL-PCBs.

Results and conclusions

The validation study resulted in accordance with the performance requirements fixed

by ex Commission Regulation EU 589/2014 (current Comm. Reg. EU 644/2017).
Accuracy and precision were evaluated in the range of the maximum levels and for the sum of PCDDs, PCDFs and DL-PCBs ranged between 91 - 110 % and 4 - 8 %, respectively.
In addition, the method was accredited by the Italian accreditation body Accredia and was used for the determination of dioxins and PCBs in official samples from different types of egg farming.
All analyzed samples were compliant with the maximum limits and eggs from free-range hens showed higher levels of PCDDs, PCDFs and DL-PCBs than those found in conventionally produced eggs according to the data reported by EFSA [2].

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DETERMINATION OF MERCURY IN SEA WATERS AND BIOTA (FISH & MOLLUSCS) BY AAS & ICP-MS

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Summary: Applicazione della direttiva quadro 2013/39/UE e del D.Lgs. 172/15. Determinazione del mercurio nelle acque di mare con tecnica ICP- MS e nel biota con tecnica AAS: risultati e correlazioni tra le diverse matrici.

Keywords: direttiva 2013/39/UE, D.Lgs. 172/15, mercurio, biota

Il D.Lgs. 13 ottobre 2015 n 172, in attuazione della direttiva 2013/39/UE, fa riferimento alle sostanze prioritarie nel settore della politica delle acque; la classificazione dello stato chimico è effettuata valutando i superamenti dei valori standard di qualità di cui alla tabella 1/A del decreto stesso per la colonna d'acqua e il biota.

La ricerca e quantificazione del mercurio, considerata sostanza pericolosa prioritaria dalla normativa comunitaria, è fondamentale nell'ambito del monitoraggio marino costiero per la classificazione dello “stato chimico“ dei corpi idrici. Per il mercurio è previsto uno SQA- CMA pari a 0,07 µg/l per ogni singolo campionamento, e lo stato “non buono” scatta quando anche un solo campione supera la concentrazione massima ammissibile (CMA).

Le sfide analitiche per il monitoraggio di questo inquinante su campioni di acqua superficiale riguardano le possibili interferenze e contaminazioni, che sono i maggiori ostacoli che possono sorgere eseguendo determinazioni nel range di concentrazione dei ng/l, e l’elevata salinità delle acque marino-costiere e di transizione.

In passato nei Laboratori ARPAT AVL il mercurio di campioni con matrici fortemente saline veniva determinato mediante la tecnica di spettroscopia di assorbimento atomico dei vapori freddi (CVAAS), basato sul metodo EPA 245.7 “Mercury in Water by Cold Vapor Atomic Fluorescence Spectrometry” in parte modificato. Questo tipo di determinazione presenta alcuni limiti, tra cui l’elevato rischio di contaminazione legato alla preparazione preventiva delle aliquote e all’utilizzo dei reagenti. Per questo motivo si è ritenuta opportuna la messa a punto di un procedimento per l'analisi diretta del mercurio nelle acque di mare utilizzando la tecnica ICP-MS in combinazione con una diluizione in linea con Argon Gas.

L'effetto matrice in ICP-MS può significativamente ridurre la sensibilità del sistema, rendere inaffidabile la risposta dello standard interno, rendere necessari frequenti cicli di manutenzione a causa del blocco dei coni e del nebulizzatore ed esporre i campioni al rischio di contaminazione a causa della necessità di diluizione "a banco".

La metodica ottimizzata ovvia ai problemi sopracitati attraverso l'implementazione del sistema (ICP MS) in uso con:
- *ARGON Gas Dilution Kit* (AGD): si tratta di dispositivo che previene la necessità di diluizione manuale preventiva dei campioni e consente ad un quantitativo inferiore di "matrice" di arrivare all'interfaccia, permettendo di operare per periodi più lunghi senza necessità di interventi di manutenzione sui coni;
- *Argon Nebulizier Gas Humidifier*: trattasi di un dispositivo che evita l'eccessiva deposizione di matrice salina sui coni, preservandone la funzionalità; facilita, inoltre, la ionizzazione degli elementi ad elevato potenziale di ionizzazione aumentandone il segnale per il recupero fino a raggiungere il limite più basso (70%).
Al fine di stabilire la capacità del sistema di fornire valori accettabili di precisione e recupero, sono stati valutati i principali parametri statistici del metodo, con analisi di CRM (ORMS-5 26.2 ±1,3 ng/l), *matrix spikes, matrix spike duplicates* e partecipazione a proficiency test (metodo: "*APHA standard methods for examination of water and waste water 22st ed 2012 3125*").
I risultati ottenuti dimostrano che la risposta dello standard interno non viene influenzata in modo significativo dalla matrice del campione mantenendo un livello di accuratezza eccellente come dimostrato dalla determinazione del recupero degli *spike*; l'esattezza è stata invece verificata con la performance del laboratorio nel circuito Quasimeme (|Z|score < 2) e nel recupero dell'ORMS.
Nel 2017 ARPAT, in aggiunta al consolidato monitoraggio del mercurio nelle acque e nei mitili (*Mytilus galloprovincialis*), ha iniziato, a livello sperimentale, il campionamento e la determinazione di sostanze pericolose nel biota, secondo le linee guida ISPRA 143/2016 realizzate per il monitoraggio delle sostanze prioritarie del decreto 172/2015.
Sono stati analizzati in totale 35 campioni di fauna ittica, prevalentemente *Liza Ramada* e *Leuciscus Cephalus* per l'ambiente marino costiero, ed è stata messa a punto la fase di preparazione del campione allo scop di rendere il materiale il più omogeneo possibile.
La fase preparativa messa a punto prevede che il campione congelato venga sottoposto ad omogeneizzazione in modo da ottenere aliquote rappresentative di massa idonea da sottoporre ai trattamenti successivi.
Questo passaggio si rende necessario sia nel caso di campioni di grandi dimensioni come i pesci, sia nel caso di pool di piccoli campioni come molluschi bivalvi e pesci di ridotte dimensioni, che non vengono analizzati singolarmente come previsto dal paragrafo 1.2.5 SCELTA DEL TESSUTO PER L'ANALISI DEL CONTAMINANTE, "*PRASSI ATTUALE DEI PROGRAMMI DI MONITORAGGIO IN CORSO"* delle Linee Guida ISPRA 143/2016.
La fase di triturazione e omogenizzazione è stata diversa a seconda del tipo di biota trattato. I mitili sono stati omogenizzati con un trituratore ad immersione, mentre i pesci sono stati ridotti in pezzi, triturati ed omogenizzati con l'ausilio di ghiaccio secco in un m**ulino a coltelli**; i campioni sono stati successivamente liofilizzati in piastre di plastica e conservati in essiccatore.

La determinazione del mercurio è stata effettuata sul liofilizzato mediante spettroscopia atomica di assorbimento con l'analizzatore automatico DMA-80 Tricell Milestone (Method EPA 7473 rev.0 del 2007).

I risultati ottenuti hanno evidenziato la correlazione tra la concentrazione dell'inquinante mercurio nelle diverse matrici biota e acque mostrando, per quanto riguarda lo stato chimico basato sui dati di acqua e biota, il mancato conseguimento dello stato buono.
Il biota presenta superamenti dello standard ambientale per il mercurio in tutte le stazioni monitorate.

MONITORAGGIO DEL TRIBUTIL STAGNO CLORURO IN TOSCANA: METODOLOGIA E RISULTATI

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Summary: *Applicazione della norma EN ISO 17353 e accreditamento del metodo di analisi di Tributil stagno cloruro (TBT) in acque superficiali mediante derivatizzazione con sodio tetrakis (4-fluorofenil) borate (TAS) ed analisi gas cromatografia in triplo quadrupolo (GC-QQQ).*

Keywords: *TBT, Acque superficiali, GC-QQQ*

Il tributilstagno (TBT), impiegato solitamente come cloruro, è un componente attivo di alcuni tipi di pitture antivegetative ampiamente utilizzate fino all'inizio del 2000.
A livello normativo, in Europa, il bando del TBT è sancito dal Regolamento (CE) n. 782/2003 mentre l'obbligo di monitoraggio di tale sostanza nelle acque superficiali è riportato nella Direttiva 2000/60 / CE.
Quest'ultima fissa gli standard di qualità ambientale (SQA) per "sostanze prioritarie" e "altri inquinanti", riportando per il TBT catione un SQA pari a 0,0002 µg/L per ogni tipo di acqua superficiale.
I composti tributilstagno non sono volatili, pertanto l'analisi in GC richiede uno stadio di derivatizzazione del campione che, nei metodi più recenti, viene effettuato generalmente "in situ" (ad esempio con i reagenti di boro).
Nel laboratorio ARPAT-AVL, è stato sviluppato un metodo di analisi del tributilstagno su campioni di acqua (marina e non) basato su una derivatizzazione diretta e spettrometria di massa tandem, applicando alcune modifiche alla norma EN ISO 17353.
Il campione da sottoporre ad analisi viene addizionato di una idonea quantità di materiale di riferimento isotopicamente arricchito (TBT D27) e sottoposto ad estrazione liquido/liquido con diclorometano.
Previa evaporazione del solvente, l'analita estratto, viene derivatizzato con una soluzione metanolica di sodio tetrakis (4-fluorofenil) borato (TAS).
La soluzione metanolica viene nuovamente estratta con diclorometano, e dopo evaporazione a piccolo volume, analizzata in GC-MS /MS (QQQ).
Il metodo consente il raggiungimento di un limite di quantificazione (LOQ) di 0,0001 µg/L in conformità a quanto richiesto dalla Direttiva 2009/90.
I principali parametri statistici, valutati mediante la partecipazione a test internazionali di competenza e attraverso un processo di validazione interna, hanno consentito l'accreditamento della metodica a Norma UNI/EN/17025 nei primi mesi del 2018.

POLYCYCLIC AROMATIC HYDROCARBONS AND RELATIVE CORRELATION WITH PM LEVELS AND INORGANIC FRACTION IN APPLE FRUITS: A PRELIMINARY STUDY IN MOLISE REGION

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Summary: *the presence of PAHs in the fresh fruit pulp in season, with particular attention to apples and pears, is related to the levels of PM determining experimentally in the harvesting areas. Two analytical protocols for the extraction and determination of pollutants are developed and compared.*

Keywords: *PAHs, PM, Fruit*

Introduction

Even if Polycyclic Aromatic Hydrocarbons (PAHs) are produced by both natural and anthropogenic pathways, the anthropogenic activities generally release much greater amounts to the environment [1]. PAHs, a compound class originated from combustion, coke production, oil derivates and high temperature industrial processes, are considered as Persistent Organic Pollutants (POPs) according to the Stockholm Convention. In many studies of contamination, they have been found in air, water, food and soil. Among the different PAHs, the U.S. Environmental Protection Agency (U.S. EPA) has identified 16-priority pollutant PAH compounds. There is evidence that some PAHs are carcinogenic, mutagenic and toxic. Monitoring of the PAHs in the environment is important in the evaluation of risk to the health of organisms as well as their determination in foods and beverages is important for the human health [2]. In this work the presence of PAHs in fruit has been related to atmospheric particulate levels. In particular, two analytical methods for the determination of PAH in fresh seasonal fruit were studied and compared. The extraction of the PAHs from the real sample was carried out using two analytical techniques studied in our laboratory for years: the Dispersive Liquid-Liquid Micro-Extraction (DLLME) and the Solid-Liquid Extraction (SLE). Between the two methodologies the DLLME was chosen due to its reliability. The analytical determinations were carried out in both cases using gas chromatography combined with an ion trap mass spectrometer, using a GCUltra PolariQ of TermoFischer Scientific.

Experimental

The atmospheric particulate (PM_{10}) was measured in two different zones, a low-traffic peripheral and one near traffic-intensive road. The PM_{10} measurements were performed by means of Smart Sampler analyzers by FAI Instrument (Fonte Nuova, Rome, Italy) (Figure 1), powered by a photovoltaic panel. The sampler filters have

been conditioned according to the specifications reported by the parent company. The sampling was carried out in the summer period, between mid-July to mid-September for a total of 60 days. Sampling was performed over 24 hours. PM_{10} particulate was gravimetrically measured.
The DLLME technique consists in a liquid-liquid extraction with the use of a small amount of extraction solvent. The traditional DLLME, present in the literature, simultaneously uses a dispersing solvent in addition to the extraction solvent, to facilitate the formation of an emulsion inside the solution. In our laboratory, for some time now, we have been developing methods that use mechanical energy supplied externally instead of the dispersing solvent, using a rotating stirrer and an ultrasonic bath [3,4]. The DLLME consists of four steps: preparation of the solution, dispersion formation, centrifugation and injection into the gas chromatograph. All phases of the analysis are studied and experimentally set up. In particular, extraction solvent, pH of the solution, dispersion formation times and chromatographic conditions are studied.

Results

The results obtained experimentally show significant recoveries, good reproducibility and limits of detection and quantification according to the mass spectrometric standards. In particular, using this method, good recoveries between 63 % and 105 % (Figure 2) are obtained, a reproducibility with an RDS of less than 18 %, and LOD and LOQ below 0.3 ppb and 1.1 ppb, respectively, are achieved. The DLLME extraction is a valid alternative to other expensive and long-time analytical preparation steps.
About PM_{10}, although the measurements are still running (long all the summertime), the levels seem to be related to sub-rural areas (about 25-30 µg m^{-3}) as well as the PAHs does not preliminarily show any significant levels in the matrices investigated.
The future steps of this study will regard the application of such methodology to a large number of sample and simultaneously to enlarge the matrices to be analyzed (e.g., other fruits without peels) for obtaining a single easy analytical procedure to be applied in every cases.

Figure 1. Smart Sampler

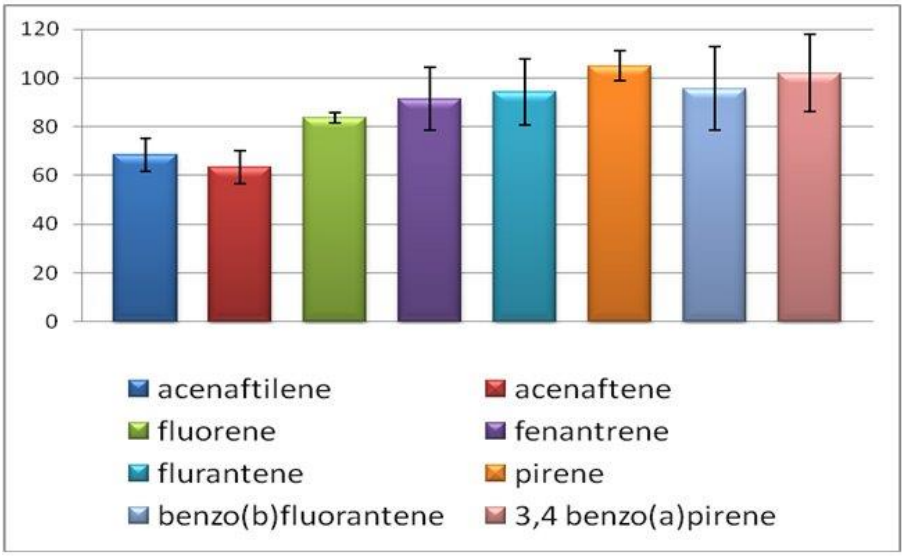

Figure 2. Percentage recovery of the extracted analytes using the DLLME technique

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A MODIFIED EPA METHOD FOR SIMULTANEOUS DETERMINATION OF CHLORINATED ORGANIC POLLUTANTS IN SOIL AND SEDIMENTS BY GAS CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Summary: *In this paper, a new method for simultaneous determination of polychlorinated dibenzo-p-dioxins/ furans, PCB and PBDE is shown. The methodology has been applied to the analysis of these compounds in soil and sediments, sampled from contaminated areas. Distribution analysis of the chlorinated congeners allows to identify possible sources of contamination.*

Keywords: *Accelerated Solvent Extraction, GC-MS tandem, Organic Pollutants*

Introduction

Epidemiological studies show that exposure to specific persistent organic pollutants (POPs) such as polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF), polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) is strongly associated with negative interferences on endocrine systems and on alterations of reproductive physiology. Therefore, the determination of these compounds in the environment is of great importance from the ecotoxicological point of view. Accurate determination of PCDD/F, PBDE and PCB in complex matrices is a challenge because of their low expected concentrations in the range of ppb to sub-ppb. For the complexity of environmental matrices, analytical determinations often require long time periods of analysis and several stages of extraction/ preconcentration, purification, etc in order to eliminate interferences. These procedures do not fit in extensive environmental monitoring programs, where it is necessary to analyze a large number of samples at a time not excessively long. In this paper, the official EPA methods [1-2] for chlorinated pollutant analysis has been evaluated and modified to provide a faster and more reliable alternative analytical method for simultaneous determination of interest compounds in soil and sediments with a single extraction.

Experimental

Accelerated Solvent Extraction (ASE) has been used for simultaneous extraction of organic compounds. The most favorable extraction conditions proved to be n-hexane as the extraction solvent, temperature of 120 °C, pressure of 1500 psi and three static cycles in each case. Purification of the extract was accomplished by automated Power-Prep™/Sample Clean-up system. The analyses were then performed by using

gas chromatography coupled to triple quadrupole mass spectrometry. Utilizing triple quadruple mass spectrometry under positive EI with multiple reaction monitoring (MRM) mode, greatly enhances the sensitivity and selectivity of detection, compared to selective ion monitoring (SIM) mode. The GC-QqQ(MS/MS) sensitivity, lower than that of GC-HRMS, is good enough (LODs in the down to low pg levels) to detect the normal concentrations of these compounds in environmental samples.

Results

The obtained analytical results demonstrate excellent recoveries for the various congeners, comparable to those of official methods and detection limits useful for the analysis of real matrices. The performance of the method was evaluate by calculating the recoveries, reproducibility and detection limits for the various congeners. Recovery tests were performed by analyzing non-contaminated matrices in triplicate to which the labeled standards were added. Under the optimized conditions, the recoveries of all targets are comparable with those recommended by US EPA method 1613 B: the repeatabilities were 20% or less. The quantification limits (LOQ) have been calculated on the order of pg/g d.w. The method was then successfully applied to the determination of chlorinated compounds in soil and sediments samples from contaminated sites. The evaluation of the distribution of the various congeners (Fingerprint method [3-4]) has allowed to obtain information on the origin of the contamination.

Conclusions

The applicability of ASE extraction combined with GC-MS triple quadrupole for the determination PCDD/F, PCB and PBDE in soil and sediments was demonstrated. Satisfactory validation parameters including linearity, LOQ, were obtained, demonstrating the feasibility of the method. The analysis of the distribution of PCDD / F and PCB congeners in the various samples provides useful indications on the identification of contamination sources. With regard to PCDD and PCDF profiles, for example,the prevalence of octa-chlorinated dioxin (OCDD) has made it possible to identify the most likely sources of contamination in the combustion processes (waste incineration, engine exhaust or industrial emissive processes). This simple and sensitive methodology is expected to provide a new tool for monitoring toxic chlorinated compounds in environmental matrices.

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DETERMINATION OF ESTROGENIC ENDOCRINE DISRUPTORS AT pg L^{-1} LEVELS IN WATER SAMPLES, ACCORDING TO DECISION 2015/495/EU

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Summary: *In this research paper, we report a method able to detect estrogenic endocrine disruptors (17-α-ethinylestradiol, 17-β-estradiol, Estrone) at pg L^{-1} levels in superficial and underground water samples. The method is based on Off line SPE Extraction followed by Online Phase Extraction and HPLC-MS/MS determination. The method was attested able to measure steroidal-estrogenic hormones at trace levels, especially we achieved limits of 0.035 ng L^{-1} for 17-α-ethinylestradiol and 0.1 ngL^{-1} for estrone and 17-β-estradiol; limits set by the 2015/495/EU and 2018/840/EU Directives.*

Keywords: *online SPE concentration, endocrine disruptors, watch list*

Introduction

The Watch List (WL) mechanism has been introduced by Environmental Quality Standards Directive (EQSD, Directive 2008/105/EC, amended by Directive 2013/39/EU). It has been designed to gather sufficient, high-quality monitoring data to support the prioritisation of pollutants under the Water Framework Directive.
The requested quantification limits by the Decision 2015/495/EU (maximum reporting limits) are set at 0,035 ng L-1 for EE2 and 0,4 ng L-1 for E1 and E2, very challenging, even with the most recent innovative analytical equipment, and need an appropriate improvement of the presently available methods [1,2].
The present study deals with the development and validation of the method including evaluation of matrix effect, trueness and precision.

Experimental

1L of sample, with the marked standards of all three sought analytes, is passed on the previously conditioned SPE OASIS HLB column, subsequently dried under nitrogen flow and eluted with 10 mL of methanol. The methanol extract is reduced to small volume and then reconstituted with 10 mL of milliQ water.
5mL of sample are then injected into the system and transferred over the Strata C18 column. The analytes are then eluted in counterflow and finally separated by the Kinetex EVO C18 column.
The determination of the analytes was performed by means of Multiple Reaction Monitoring (MRM) acquisition in negative ionization mode.

Results

The validation of the method was carried out according to the guidelines of the SANTE directive.

The *linearity* of the method was verified by constructing six-point lines, replicated several times. Linearity was considered sufficient when the correlation coefficient was higher than 0.99 and residues less than 20%.

Accuracy and *repeatability* were evaluated by analyzing three different surface water samples fortified at three concentration levels (0.035, 0. 28 and 0.56 ng L -1) for 17α-ethynyl-estradiol and (0.070, 0.56 and 1.12 ng L-1) for the estrone and 17β-estradiol. For all compounds, the percentage of recovery was found to vary between 90 and 110%.

The *quantification limits (LOQ*) of the method were determined using the standard deviation of replicated analysis of fortified samples at low concentrations.

Table 1 shows the results obtained for these performance parameters.

Table 1: Method detection limits (MDLs), linearity range, R^2, Recovery, Repeatability parameters evaluated at LOQ

Compound	MDL (ng L^{-1})	Linear range (ng L^{-1})	R^2	Recovery %	Repeatability CV %
17-α- ethinyl-estradiol	0.035	0.035 – 1.12	0.999	96.7	10.5
17-β-Estradiol	0.1	0.07 - 2.24	0.999	99.9	17.20
Estrone	0.1	0.07 – 2.24	0.999	105.9	17.43

Finally, to verify analytical method, robustness and matrix effects were assessed for each compound by comparing slope of calibration curves obtained in milli Q and superficial water samples. Three replicates for each level were used to obtain calibration curves, and robustness was studied by ME (Matrix Effect) index. ME was evaluated according to the following equation (1):

$$ME\ \% = \frac{m(sample)}{m(standard)} * 100 - 100 \quad (1)$$

where m (sample) is the slope of the calibration curve obtained in matrix and m (standard) is the slope of the calibration curve in solvent; accordingly, ME values < 0 indicate ion suppression, whereas ME values > 0 indicate ion enhancement.

The ME index, calculated for each analyte in mineral water and real surface water, is shown in Table 2.

Table 2: Matrix Effect Index for all analyte

Analyte	ME Index
17-α- ethinylestradiol	17.08
17-β-Estradiol	-10.82
Estrone	-8.17

As reported in literature [3], ME index value in the range of ± 20, are considered to have a negligible influence on the performance of an analytical method. Concerning to analyte studied ME index is less than ± 20, and at this regard, method developed can be considered robust. By considering that generally SPE procedures minimize matrix effect, the ME index within ± 20 can be ascribing to the use of SPE off line coupled whit SPE on line

Conclusions

In this study, an analytical method was developed for the detection of estrogens in ground and surface waters through a double extraction (off-line + ON LINE SPE) followed by analysis in MS / MS.

This method is able to quantify EE2 at the concentration required by the European Community with the 2015/495 / EU and 2018/840 / EU Directives (0.035 ng L^{-1}).

The matrix effect was also evaluated and no significant differences were observed between surface water samples and mineral water samples.

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HPLC-ESI(+)-MS/MS METHOD FOR SCREENING AND TRACE LEVEL DETERMINATION OF PHARMACEUTICALS IN AQUEOUS ENVIRONMENTAL SAMPLES

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Summary: *The application of LC–MS/MS operating in the SRM mode provided good limit of quantification and selectivity for the determination at trace concentration levels of six pharmaceuticals, amoxicillin, metformin, omeprazole, carbamazepine, erythromycin and clarithromycin, occurring in environmental samples.*

Keywords: *Liquid chromatography–tandem mass spectrometry, pharmaceuticals, selected reaction monitoring*

Introduction

During the last three decades, the impact of chemical pollution has focused almost exclusively on the conventional "priority" pollutants. However, the growing use of pharmaceuticals worldwide, classified as the so-called emerging contaminants, has become a new environmental problem, which has awakened great concern among scientists in the last few years. Wastewater treatment plants (WWTPs) are major contributors of pharmaceuticals in the environment. Due to their high consumption, pharmaceuticals along with their metabolites are continuously introduced to sewage waters, mainly through excreta, disposal of unused or expired drugs or directly from pharmaceutical discharges [1]. For this reason, pharmaceuticals may be able to cause the same exposure potential as persistent pollutants, since their high transformation and removal rates can be compensated by their continuous input into the environment. Consequently, there is a growing need to develop reliable analytical methods, which enable their rapid, sensitive and selective determination in environmental samples, at trace levels. In this work, for the first time, an HPLC solvent gradient, based on tri-phasic system, coupled with a selected reaction monitoring (SRM) MS-scan mode, were presented for simultaneous analysis of amoxicillin, metformin, omeprazole, carbamazepine, erythromycin and clarithromycin.

Experimental

LC-MS/MS analysis was performed by using a reverse-phase Accucore-150-C18 (150 x 4.6 mm i.d., 2.6 µm) with a pre-column (10 x 4.6 mm i.d., 2.6 µm, ThermoFisher Scientific, USA), coupled with ESI–LTQ mass spectrometer (ThermoFisher Scientific, USA). MS analysis was performed in positive ion mode.

Results

To optimize the chromatographic separation, several preliminary experiments were performed, testing different mobile phases consisting of acetonitrile as an organic phase and water with formic acid ad additive. The optimal separation of 6 compounds detected in positive ion mode was achieved using tri-phasic system consisting in water, 0.1% aqueous formic acid and acetonitrile. The optimization of MS parameters (cone voltage and collision energy) was performed by flow injection analysis (FIA) for each compound. Representative chromatograms of a l mg/L standard mixture of the compounds analyzed and the comparison with other biphasic solvent gradient are illustrated in Figure 1. The transitions *m/z* 398>381 for amoxicillin, *m/z* 130>60 for metformin, *m/z* 346>198 for omeprazole, *m/z* 237>194 for carbamazepine, 734>573 for erythromycin and 748>590 for clarithromycin, were recorded in SRM mode for identification and quantification.

Linearity, precision, limits of detection and quantification, recovery and uncertainty were considered as the criteria for the validation of the analytical methodology developed. Calibration curves were generated using linear regression analysis and over the established concentration range (0.001–200 μg/L) gave good fits ($R^2 > 0.993$). Intra- and inter-day precision, were less than 11.91% and 14.86%, respectively. LOD ranged from 0.0001 μg/L to 0.5114 μg/L and LOQ changed between 0.0004 μg/L and 1.6955 μg/L). Recoveries achieved for all target compounds ranged from 50 to 116%.

Conclusions

The validation results, obtained for LC-MS/MS method, provided a reliable and robust tool that can be used for routine analysis of pharmaceuticals in aqueous samples.

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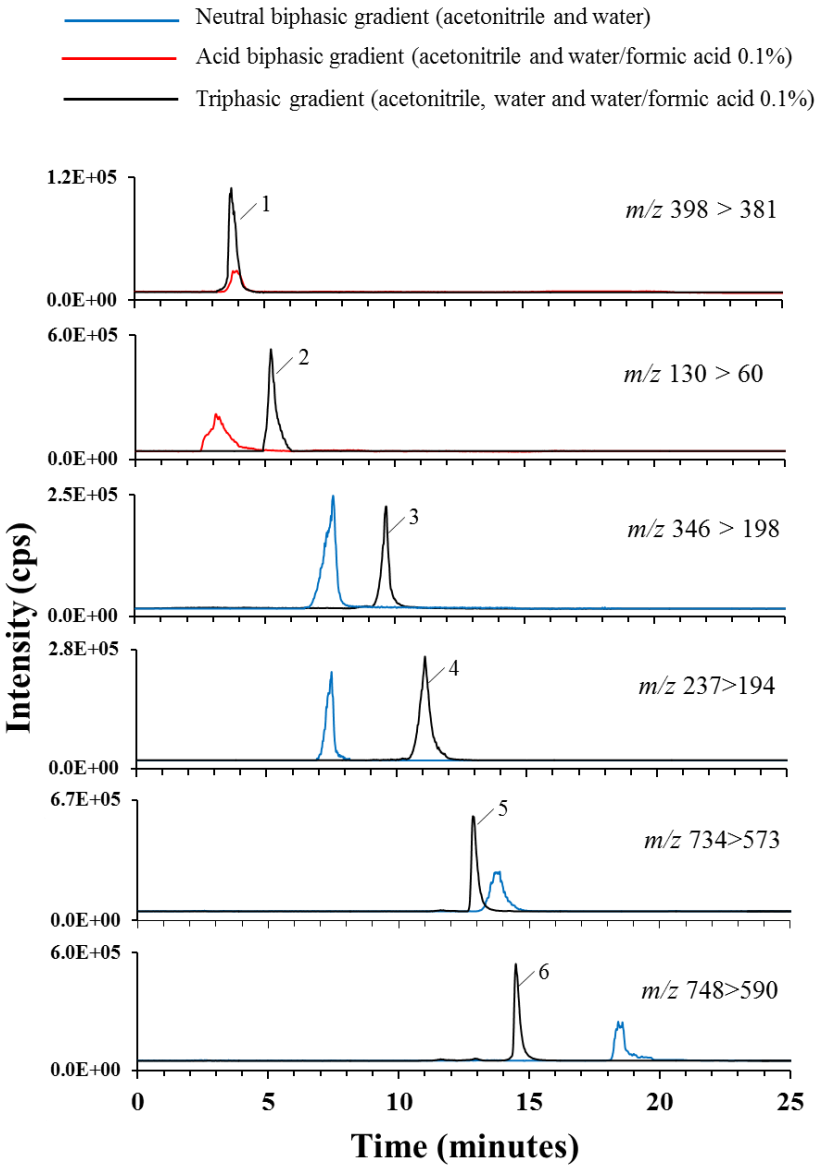

Figure 1. Representative XIC of a l mg/L standard mixture of target pharmaceutical: (1) amoxicillin, (2) metformin, (3) omeprazole, (4) carbamazepine, (5) erythromycin and (6) clarithromycin.

GLYPHOSATE AND AMPA: METHODOLOGY & RESULTS

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A method based on ISO 16308:2014(E) and USGS Techniques and Methods 5–A10 (derivatization with FMOC, preconcetration with SPE and HPLC/MSMS) was developed with some modifications.

The application of the previous mentioned changes and mainly the use of UHPLC-HRMS/HCD (Ultra High Performance Liquid Chromatography-High Resolution Mass Spectrometry/Higher Collisional Dissociation) in positive ionization mode, permits an identification in compliance with Decision EU 657/2002 of the analytes and the reaching of a limit of determination (LOD) of 0.005µg/L in superficial and groundwater.

The isotopic diluition with stable-isotope labeled AMPA and glyphosate allows a quantitation range for the method from 0.02 to 0.5 µg/L without use of calibration curves.

All statistical parameters needed to the validation process were determined and the method was accreditated according to UNI-EN-ISO/IEC17025:2005 in april 2016.

The results of 240 water samples collected in Tuscany Region during the first eight months of 2016 were reported in comprehensive maps of the sampling points.

The data show that AMPA and Glyphosate are usually detected together and occur widely in the aquatic environment of the Region with concentration range from 0.05 to 65 µg/L for AMPA and from 0.05 to 24 µg/L for Glyphosate.

AUTHOR INDEX