

# 1<sup>st</sup> NORMAN workshop on analysis of problematic compounds

## *How can we analyse very polar and hardly-ionisable compounds*

Reason for the workshop:

As producers aim to reduce the production of persistent compounds, the number of low molecular weight compounds (polar and non-polar) ending up in the water cycle is constantly increasing. Current analytical techniques, such as reversed phase HPLC, cannot separate or retain the very polar compounds. Hydrophilic Interaction Chromatography (HILIC) columns are a promising alternative chromatographic technique for the separation and determination of polar compounds. However, using these types of columns is not always straightforward and their optimisation and behaviour differ significantly from normal phase chromatography.

As regards, non-polar, hardly ionisable compounds, they cannot be determined effectively by conventional ionisation methods such as ESI. Alternative ionisation techniques such as Atmospheric Pressure Photo Ionisation (APPI), can therefore be used in conjunction with LC separation, to expand the applicability of the LC-MS techniques. This method of ionisation, however, is still in its infancy compared to ESI.

The purpose of this two-day workshop was to share the information currently available on the analysis of very polar and non-polar hardly ionisable compounds. Both HILIC and the ionisation technique APPI were discussed.

Summary of the workshop:

Over 30 interested participants were present at the workshop in Rhodes (Greece) to discuss the advantages and disadvantages of HILIC and APPI. Nine presentations were given on the progress made with these two techniques. The presentations sparked a lively discussion on both topics. The scientists shared their experiences and discussed the advantages offered by these new techniques as well as the possible drawbacks and problems. The most important findings for both techniques are summarised in this document. As a result of the discussion on HILIC, the wish was expressed by several experts to organise a collaborative trial to test the methods among different laboratories for polar organic compounds.



## Programme of the Workshop

**Tuesday 1 September:****HILIC Session** (Chair: Patrick S. Bäuerlein)

- 09:00** Arrival and Registration
- 09:30** Basic theory and method development and optimization of HILIC methods (Nikolaos Thomaidis)
- 10:00** HILIC as a tool in environmental research. What is the potential? (Annemieke Kolkman)
- 10:30** HILIC-QTOF-HR-MS/MS for the orthogonal screening and identification of polar micropollutants in environmental samples (Anna Bletsou)
- 10:50** Orthogonal identification of biotransformation products by HILIC-QTOF-MS/MS (Aikaterini Psoma)
- 11:10** Study of the metabolism of zebrafish (*danio rerio*) embryo exposed to triclosan and benzotriazoles by HILIC-TOF-MS (Dimitrios Damalas)
- 11:30** Panel discussion + Coffee
- 12:30** Lunch
- 13:30** Hydrophilic interaction liquid chromatography coupled to mass spectrometry for targeted and non-targeted metabolomics (Marja Lamoree)
- 14:00** RPLC/HILIC/API-MS: polarity extended analysis for organic molecules in water bodies (Sylvia Grosse)
- 14:30** Panel discussion + Coffee

**APPI Session** (Chair: Nikolaos Thomaidis)

- 15:00** APPI in environmental chemistry (Pim de Voogt).
- 15:30** Development and optimization strategy of LC-APPI-MS methods for the determination of halogenated compounds in environmental matrices (Nikolaos Thomaidis).
- 16:00** Advantages and challenges of APPI for the analysis of environmental matrices (Juliane Hollander)
- 16:30** Panel discussion + Coffee
- 18:00** End of Day 1
- 19:30** Participants' dinner

**Wednesday 2 September**

- 09:00** Discussion and Workshop Wrap-up
- 11:00** **End of the workshop**

### Most important findings for APPI

- APPI is a good ionisation technique for fullerenes, (heterogeneous) polyaromatic hydrocarbons (PAC), UV stabilisers and polyhalogenated compounds.
- The experience is that APPI in positive mode is more prone to interference than positive ESI.
- In negative mode APPI generally provides “simpler and cleaner” mass spectra than positive APPI or ESI.
- APPI is a complementary technique and can fill the gap other ionisation techniques leave (see figure).

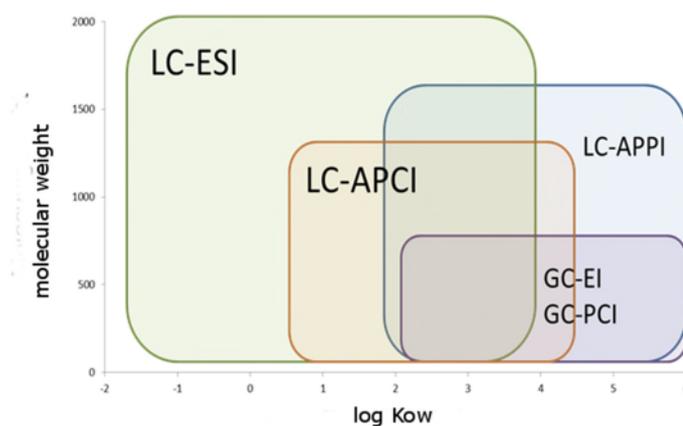


Figure 1: APCI = Atmospheric Pressure Chemical Ionisation, EI = Electron Ionisation, PCI = Positive Chemical Ionisation

### Most important findings for HILIC

- HILIC is a powerful and promising tool for the analysis of polar transformation products and in metabolomics.
- It is complementary technique to reverse phase chromatography (RP) and provides additional information.
- Method development for HILIC is not as straightforward as it is for RP.
- Parameters (pH, T, composition of the mobile phase, in-vial composition and equilibration time) have a greater influence on the performance of the method compared to RP.
- It can be useful for both non-target and target HRMS screening.

### Collaborative trial for the analysis of polar compounds

As a result of the discussion on HILIC, the wish was expressed to organise a collaborative trial. For this trial all NORMAN members will be invited as well as interested other parties. The primary goal of this trial will be to evaluate the different methods in the laboratories and compare them. Each laboratory is free to choose its own method(s). This means that this trial is not restricted to only HILIC columns, but other columns (such as polar embedded columns) or techniques are expressively allowed. This approach will make it possible to evaluate whether other techniques are also suitable candidates for the analysis of polar compounds.

To summarise: During the workshop the following issues were agreed:

- For this trial between 10 and 20 compounds will be used
- Each laboratory can choose the analytical method
- KWR, University of Athens and the TU Munich will compile a list with relevant compounds
- These compounds should be mostly pharmaceuticals and metabolites
- The aqueous matrix will be surface water
- The samples should be analysed quantitatively and qualitatively
- The laboratory costs for this trial will be paid by the participants
- A proposal will be submitted to Norman to ask for financial support for the organisation of this trial
- All Norman members and interested parties can take part in this trial
- The data from this trial will be used for a scientific publication

So far the following institutes have expressed their interest in participation:

- KWR Water Cycle Research Institute (The Netherlands)
- UFZ Leipzig (Germany)
- Technical University Munich (Germany)
- University of Athens (Greece)
- Recetox (Czech Republic)
- University of Antwerp (Belgium)
- EAWAG (Zwitzerland)
- IVM (The Netherlands)
- Veolia (France)
- UvA (The Netherlands)
- Ineris (France)
- Lancaster University (United Kingdom)
- NCSR Demokritos (Greece)