RESEARCH – DEVELOPMENT REPORT

Project title: Improvement of methods and techniques for identification and determination at nanoscale of steroid structures contaminants in food

Stage I

Stage duration: June 2008 – Nov. 2008

The objectives of the stage:

- To train the Romanian partner in Budapest, ELTE University, in high resolution chromatographic determination;
- To document the Hungarian and Romanian partners regarding the methods and techniques applied to analyze and fingerprint the wines, to determine the raw material quality (grapes) of different variety and to identify the pesticides and PCB in grapes, at SC JIDVEI SRL
- To elaborate new modern methods which uses high performance analytical techniques as: high performance liquid chromatography coupled with mass spectrometry LC-MS/MS, gas chromatography coupled with mass spectrometry (GC-MS, GC-MS/MS (with ionic trap)) to determine the contaminants with steroid structure from different complex matrices, at nanoscale level;
- To use solid phase extraction techniques SPE, with different cartridges: DSC-18, 500 mg and ENVI ChromP, 200 mg (Sigma-Aldrich, St. Louis, MO, USA), Oasis, HLB 200 mg, Oasis Max 60 mg, Oasis, MCX 60 mg, Waters (Milford, MA, USA), Strata-X 200 mg (Phenomenex, Torance, CA, USA);
- To use automatic solid phase microextraction techniques SPME with the help of CombiPal CTC autosampler connected to GC-MS;
- To use automatic extraction techniques SBSE (stir bar sorbtive extraction), (Twister-Gerstel) with the help of CombiPal CTC autosampler connected to GC-MS;
- To realize two stage derivatization methods: esterification and acylation – oximation and silylation.

Activities involved:

A. I.1 Official trip to Science University Eötvös Loránd, Budapest

Between 17-27.11.2008, the Romanian team formed from chem. Mirela Miclean and phys. Dorina Simedru visited the Chemistry Institute of Science University Eötvös Loránd, Budapest. With this occasion the contaminant chemical substances were established:

- cholic acids – steroid structure contaminants – in liquid matrix;
- drug compounds: ibuprofen, naproxen, ketoprofen and diclofenac;
- steroid compounds: estrone, β-estradiol, cholesterol;
- organochlorine pesticides (POC);
- polychlorinated biphenyls (PCB).

The members of the Romanian team also participate to the experiencing of some of the methods to determinate the steroid colic acids (colic acid, litocholic acid, chenodeoxycholic acid, ursodeoxycholic acid, 3–hydroxy, 7-ketocholanic acid, dehydrocholic acid), of some hydrosoluble residues (including pesticides, estrone, β-estradiol, cholesterol) of some drug residues (ibuprofen, naproxen, ketoprofen and diclofenac) in liquid matrix. The investigated methods were very recent, involving two steps derivatization: oximation and silylation, detection by gas chromatography coupled with mass spectrometry and ionic trap (GC-MS/MS), allowing multicomponent residual analysis in a single sample injection.

The sample extraction method was solid phase extraction (SPE), a method relatively new, with multiple advantages: allow simultaneous the separation and the concentration of the analytes, it doesn't use solvents for extraction (only small volumes to elute), allows a very specific separation of the analytes. Different SPE cartridges were used to choose the proper solvent in order to obtain better analytes recovery.

The Romanian team participated at Ekol Group, located at Chemistry Institute of Science University Eötvös Loránd, Budapest, to a training for the following instruments: high performance liquid chromatograph (HPLC, 1200 Series Agilent Technologies), liquid chromatograph coupled with mass spectrometer, triple quadrupol (LC-MS/MS Triple Quad, 6410 Series Agilent Technologies with HPLC, 1200 Series, Agilent Technologies),

gas chromatograph coupled with mass spectrometer (GC-MS, 7890 Series, Agilent Technologies) with CombiPal CTC for headspace analysis, SBSE (stir bar sorbtive extraction) (Twister, Gerstel), SPME (solid phase microextractions).

A.I.2 Receiving of the official call from the Science University Eötvös Loránd, Budapest

Between 10-16.11.2008, ICIA Cluj-Napoca was visited by the following members of the Hungarian partner team from Science University Eötvös Loránd, Budapest: Professor Gyula Záray and PhD Viktor Gábor Mihucz.

With this occasion, the two partner teams studied, investigated and established the quantitative and qualitative determination methods of the residues of different food contaminants:

- organochlorine pesticides **(**POC**);**
- polychlorinated biphenyls (PCB).

The proposed methods present multiresidual analysis and are formed from different stages: the homogenization of the sample, the dilution with different solvents, liquid-liquid extractions with different solvents or mixes of solvents and also solid phase extractions with different specific cartridges. The analytes detection can be made by gas chromatography with electron capture or mass spectrometry. Specific capillary columns and temperature programs were proposed to obtain high selectivity and sensibility.

The members of the partner teams visited S.C. Jidvei S.R.L. where they studied the production process of some variety of wines, the application procedure of the pesticides on vine and they gather grape samples from different variety of vines to analyze them by POC and PCB.

The analysis allows the multicomponent detection of POC and PCB in a single sample injection.

A.I.3 Activities foreseen in the research program of the young researcher

Beginning with December 2008, chem., Lăcrimioara Şenilă, PhD student at Faculty of Chemistry and Chemical Engineering of Babes – Bolyai University was hired at ICIA

Cluj-Napoca, as young researcher in the Romanian – Hungarian bilateral cooperation project.

The activities foreseen are:

- practicing the sample preparation techniques
    - liquid – liquid extractions
    - solid phase extractions;
    - solid phase microextractions.