FINAL REPORT

PROJECT TITLE:

Improvement of methods and techniques for identification and determination at nanoscale of steroid structures in food, CONTALIM

ROMANIAN PARTNER:

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FOREIGN PARTNER:

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GENERAL OBJECTIVES PURSUED:

- Development of modern methods using advanced analytical techniques such as: high performance liquid chromatography coupled with mass spectrometry (LC-MS) or LC-MS/MS, gas chromatography coupled with mass spectrometry (GC-MS) to determine the contaminants with different matrix structure of steroids, at the nanoscale level;
- Use solid phase (micro) extraction techniques SP(M)E;
- Using the techniques of purification and concentration before chromatographic determination;
- National regulations still no concrete methodology for the determination of these contaminants in food, linked to EU law relating to the normal accepted way. This project represents "know-how nationwide. Team partner of Romania to benefit from the experience of Hungarian partners to investigate this type of compounds with steroidal structures.

Collaboration between the two teams has led to the accomplishment of the Hungarian-Romanian project objectives. Research activity in the analysis of steroid structure of food contamination led to the development and improvement of methods for identification and detection of contaminating steroids in aqueous and biological matrices. Determination of contaminants in food was performed using chromatographic techniques by the application of advanced extraction methods, sensitive and accurate.

Chromatographic determination of steroids structures is a less method applied in Romania but with the Hungarian partner who is specialized in such determinations, a number of contaminants could be determined through a number of methods such as: solid phase extraction, solid phase microextraction, liquid phase extraction, derivatization and chromatographic analysis. The Romanian team has benefited from the experience of the Hungarian team in contaminants analysis.

The project "**Improvement of methods and techniques for identification and determination at nanoscale of steroid structures contaminants in food"** had two reporting stages.

The Hungarian team received information on the Romanian research results during the project. **Stage I** of the project had three activities, namely: *Official trip to Science University Eötvös*

Loránd, Budapest, Receiving of the official call from the Science University Eötvös Loránd, Budapest and *Activities foreseen in the research program of the young researcher.*

As part of *Official trip to Science University Eötvös Loránd, Budapest activity*, the two teams have established categories of chemical contaminants to be analyzed: colic acids (structures contaminated with steroids - in aqueous matrices), steroids compounds (estrone, ß-estradiol, cholesterol), medicinal compounds, organochloride pesticides (OCPs) and polychlorinated biphenyls (PCBs), these compounds are considered endocrine disrupting compounds.

In this activity, Romanian team members participated in testing methods of colic acid steroids determination in aqueous matrices.

The applied analysis methods were solid phase extractions (SPE) with two-step derivatization: oximation and sylilation. Also during this activity, the partner teams have analyzed soluble residue, as trimethylsilyl derivatives by gas chromatography coupled with mass spectrometry GC-MS/MS. Four anti-inflammatory drugs from aqueous samples were investigated: ibuprofen, naproxen, ketoprofen and diclofenac. The drug compounds were analyzed and quantified by GC-MS technique. SPE was the extraction method used followed by two-step derivatization: reaction with MSTFA (and hydroxil group's acetylation with MSTFA). For drug compounds analysis there were studied: derivatization studies with different silylation agents, response differences for internal and external techniques, different elution techniques, TIC and SIM, reproducibility studies of the derivatized compounds for medicinal compounds, in an area of concentration of picograms, from synthetic solutions and wastewater samples.

As part of *Receiving of the official call from the Science University Eötvös Loránd, Budapest,* there were studied and investigated the contaminants in various food samples, namely: organochloride pesticides (OCPs) and polychlorinated biphenyls (PCBs).

These compounds were analyzed due to the endocrine disrupting effect with carcinogenic, reproductive and hormonal EFFECTS. The members of both teams have proposed a method for organochloride pesticides and polychlorinated biphenyls determination in food samples. The proposed method by the two teams was: multiresidue analysis, SPE-GC-ECD(MS) or LLE- GC-ECD(MS).

In this activity, the members of the two teams visited the S.C. Jidvei S.RL., Loc. Jidvei, Alba County, where they collected and analyzed samples of grapes for chromatographic determination of organochloride pesticides and polychlorinated biphenyls, by multiresidue analysis, by a single injection in GC-ECD.

As part of *Activities foreseen in the research program of the young researcher*, since December 2008 has been employed a young researcher: chemist Lacrimioara Senila.

The first stage of the project led to goals accomplishment, namely: Romanian partner was trained to the ELTE University, Budapest, in high resolution chromatographic determination, Romanian and Hungarian partners have proposed methods for analysis of food (different types of grapes) for identification and quantification of organochloride pesticides and PCB in grapes; developed modern methods, cutting-edge, using advanced analytical techniques: gas chromatography coupled with mass spectrometry ((GC-MS, GC-MS/MS (with ion trap)) for determination of contaminants with steroid structure of different matrices, at nanoscale level; there were used solid phase extraction (SPE) tehniques, using different cartridges; there were used SBSE, automated extraction technique (stir bar sorbtive extraction) connected to GC-MS; derivatization methods have been conducted in two stages: esterification and acylation-oximation and silylation.

Phase II of the project has 3 activities in which were accomplished the objectives.

In the course of 1st activity, Visit to the Eötvös Loránd University, Budapest, the Romanian team together with the Hungarian team conducted a qualitative determination of chemical compounds with steroid structure: ► estrone, ► β-estradiol, ► cholesterol in samples with matrix aqueous. The experimental methodology was applied for the first time by the Hungarian partner team members.

The used analytical method was SPE-GC-MS/MS and involved several steps: filtration, acidification of the sample, conditioning of polymer cartridges, solid phase extraction on the cartridge, extracts evaporation, two stage derivatization: ► oximation (with hydroxylamine hydrochloride) and ► silylation (with HDMS and TFA), chromatographic determination of TMS-oxime derivatives, qualitative analysis based on retention times and modes of fragmentation of the derivatizated species and identification of information carrier large masses ions, by TIC elution mode of the ion selective fragment. Also, in this phase of the project, the determination method of the steroid hormones (estrone and estradiol) in water samples by derivatization SPE-GC/MS and two-stage (oximare and silylation) has been tested and applied. The activities encompassed also a qualitative determination of organochlorined pesticides (POC) and polychlorinated biphenyls (PCBs) by injecting pure standards and real samples under the same chromatographic conditions and comparing the retention times of analytes from the pure standards with those from sample separated by chromatography column.

Activity 2, *Receiving visit from the Eötvös Loránd University, Budapest,* consisted in quantitative determination of chemical contaminants, by carrying out calibrations for the classes of compounds established at the Hungarian partner team visit from 2008: organochlorined pesticides (OCPs) and polychlorinated biphenyls (PCBs). Quantitative analysis was performed using calibration curves and interpolating signal values of the detector on the calibration curve.

The activity included a stage of implementation and testing of the methods established with the Hungarian partner team in order to determine POC and PCBs in food samples (milk and grape). Chemical analysis consisted in the application of LLE-GC-ECD technique and included several stages: sample homogenization, extraction with organic solvents, evaporation of the solvent, extract purification, elution, evaporation of the eluate, and finally GC-ECD analysis.

In the research program of the young researcher the methodologies for sample preparation prior to chromatographic determination were developed, by performing the purification and concentration using: ► on solid phase extraction (SPE), ► microextraction on solid phase (PEMS), ► liquid-liquid extraction and their application in different analysis for determination of the structure of compounds contaminated with steroid (estrone, estradiol) and organoclorurate pesticides and polychlorinated biphenyls. The study focuses on the SPE extraction steps: conditioning (activation) of cartridge, elution through the sample cartridge, washing and elution and for extracting SPME were studied the factors affecting the process: extraction time, temperature, technique used, agitation of the sample, its volume and pH. The applicability of SPME methods in determining steroid hormones in aqueous and complex organic matrix (serum) by the technique of GC / MS, after derivatization and microextraction on fiber was studied. Water samples was taken from the Somes river and were subjected to extraction SPME with polyacrylate fiber (polar), then derivatiyed using headspace technique by exposing the fiber to MSTFA vapors. Blood samples were collected from live chickens from a farm located near Cluj-Napoca. Samples were subjected to pretreatment to remove secondary compounds, then extracted on fiber and derivatized with MSTFA; analytes of interest were separated, identified and quantified by GC / MS in SIM mode.

The project had been proposed new methods, innovative, next-generation chemical contaminants in different matrices (water, food) can help to the potable water monitoring, food monitoring on the whole technological flux (from raw material to final product) for producers and also for suppliers.