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AUTOMATED RAPID ANALYSIS FOR DIOXINS IN FOOD AND FEEDINGSTUFFS

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By the end 20th century the problem of chemical safety of food grew into a thorny complex international problem, the decision of which is impossible without joint efforts of chemists, toxicologists and technologists. This problem is part of International Program on Chemical Safety (IPCS), which is founded in 1980, and which is the joint venture of United Nations Environment Programme (UNEP), International Labour Organization (ILO) and World Health Organization (WHO). General aims of IPCS are establishment of scientific bases of risk estimation for the health of man and state of environment, conditioned by the unfavorable action of chemicals, by means of international consideration of processes as pre-condition for maintenance of chemical safety and providing of technical help to the national organs on a correct management by chemicals.

One of the first places in IPCS occupies problem of chemical safety of food how chemical safety of agricultural and food raw material and food stuff is one of basic factors, which determines the health of population and maintenance of population on the whole, in connection with that with food in the organism of man can get of foreign chemicals are xenobiotics, presenting a health hazard man.

The basic amount of xenobiotics gets in food raw material and foodstuff by chance from the objects of environment (water, soil, atmospheric air), which can contain these pollutants. To it contamination, conditioned by the use in the processes of processing of food raw material of technologies, can be added, necessary substances and materials which can be the sources of presence or formation of xenobiotics.

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Among of enormous amount of xenobiotics, being in an environment, the special danger is presented by substances, taken by Stockholm convention to the group of the so-called persistent organic pollutants (POPs, "dirty dozen"). Most danger for a man and environment among POPs present compounds which are incorporated by a general term - dioxins.

In Ukraine contamination of environment dioxins stipulate mainly technogenic-industrial sources (metallurgical industry, motor transport, thermal power-stations, cement kilns, municipal waste incenirators). It is impossible not to take into account and contamination of environment of Ukraine dioxins because of transfrontal transfer with atmospheric air. In this connection it is necessary to notice that because of insignificant volatility of dioxines capacity for the spatial moving by skyway in (vaporous) a gaseity for them is practically absent. However because of the strong sorption co-operating of dioxines with organic components of emission in the atmosphere (mainly with soot) of different productions, concentration of them in mid air considerably higher, than that which it was necessary to expect coming only from volatility of these compounds.

After freeing of dioxines in air in the adsorbed state on particulate matters (soot, volatile ash, dust) there is besieging of particulate matters on the surface of soil and plants. The besieged amounts of dioxines depend on the closeness of object which dioxines are besieged on, to the source of freeing of dioxines, type of object, weather terms and other specific parameters, such as a height of being of object above a sea level, geographical breadth of location of object and temperature.

Except the hit of dioxines in soil with the besieged particulate matters from atmospheric air, the accumulation of dioxines in Ukrainian soils can take place also as a result of application of muddy silts of sewages on the agricultural fields, flood of pastures muddy flows and as a result of antecedent the use of pesticides and fertilizers, containing the admixtures of dioxines (for example, herbicides are derivatives of chlorophenoxyalkancarbon acids, some composts).

High persorption of dioxines by soil and their extraordinary subzero solubility in water makes impossible their penetration out of soil in vegetans plants and their translocation in them and can not result in contamination the dioxines of vegetable foodstuffs and vegetable feedingstuffs.

Thus, by the main source of contamination the dioxines of vegetable foodstuffs and vegetable feedingstuffs there is precipitation from atmospheric air solid particles, containing dioxines, on the surface of plants.

Dioxines are lipophilic compounds which accumulate in fat of animals. Types of food, which contain the high concentrations of dioxines are include milk and dairies, meat and poultry, eggs, fish and adiposes. Green vegetables, fruit and breadstuffs, behave to those types of food, which are contain by the least concentrations of dioxines.

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The analysis of dioxines consists of the next basic stages: extractions, cleaning of the got extract by means of adsorption column chromatography and identification and quantitative determination of analyts with the use of combination high-resolution (capillary) gas chromatography (HRGH) with high-resolution mass-spectroscopy (HRMS)The analysis of dioxines is expensive enough as compared to determination of other chemical pollutants, that naturally is a limiting factor for the programs of monitoring.

Analysis of Dioxins

Extraction

Soxhlet/Dean-Stark
Liquid-Liquid
Micro-waves
Supercritical Fluid Extraction (SFE)
Accelerated Solvent Extraction (ASE, PLE)
Solid Phase Extraction (SPE)

Clean-up
Liquid chromatography

Analysis
HRGS/HRMS
Bio-assays

Stage of preparation of samples, which includes the stages of extraction and cleaning, is a key element at determination of dioxines. Presently there is a row of methods of analysis of different agencies and departments (EPA US et al.), by means of which it is possible to determine content of dioxines in different matrices, based on the different charts of preparation of samples.Regardless of method of analysis preparation of samples necessarily contains three stages: extraction, cleaning of the got extract and concentration of eluates after chromatographic columns.

Because of high hydrophobicity of dioxines these compounds, as a rule, accumulate and are mainly in lipophilic matrices or in lipophilic parts of analyzable matrices. In this connection the aim of the stage of extraction is a selection from the analyzable matrix of lipid fraction, which contains interesting analytes. For extraction of dioxines from solid matrices at present time mainly the next types of extraction are used: extraction by organic solvents in the apparatus of Soxhlet and accelerated solvent extraction (ASE) or pressurized liquid extraction (PLE).

A few grammes (4-5 g) of lipids, extracted from an analysable matrix, are usually needed for quantitative determination of dioxines. Limits of detection, for example, of the most toxic isomer of 2,3,7,8-TCDD (2,3,7,8 - TCDD) in different matrices make the 0,1 – 1,0 pg/g of fat (lipid). After gravimetrical determination of content of lipids fats must be deleted in an order to do possible subsequent analysis. For this purpose there is some methods: acid breaking up, saponification, use of columns with silica gel, imprergnated sulphuric acid, gel-permeation chromatography.

The stage of cleaning of the got extracts includes the use of methods of two types: **1)** liquid extraction which consists in the redistribution of having a special purpose analyts between different solvents and **2)** multi-stage successive cleaning with the use of solid phase extraction with the different mechanisms of sorption.For this aim use glass columns, filled by silica gel (including modified by sulphuric acid), alumina, florisil, carbon and sorbents for a gel-permition chromatography.On this stage of analysis the partial fractionating of analyts and their preliminary concentration is simultaneously arrived at.

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For the acceleration of processes of extraction and subsequent cleaning of extracts, containing dioxines, a few automatic systems are created. One of such systems combines liquid extraction under pressure (Pressured Liquid Extraction System (PLE), Fluid Management System (FMS), Inc., USA) and cleaning of extracts with the use of chromatography (Power-Prep™, FMS, USA). For extraction from hard matrices in this system mixture of 10% dichloromethane in n-hexane is used. For automated cleaning of the got extract high capacity disposable silica column, multiplayer silica column, alumina column and carbon column are used which are produced also by FMS.

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Pressured Liquid Extraction System (PLE),
Fluid Management System (FMS), Inc., USA

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Cleaning of Extract System, Power- Prep™, FMS, Inc., USA

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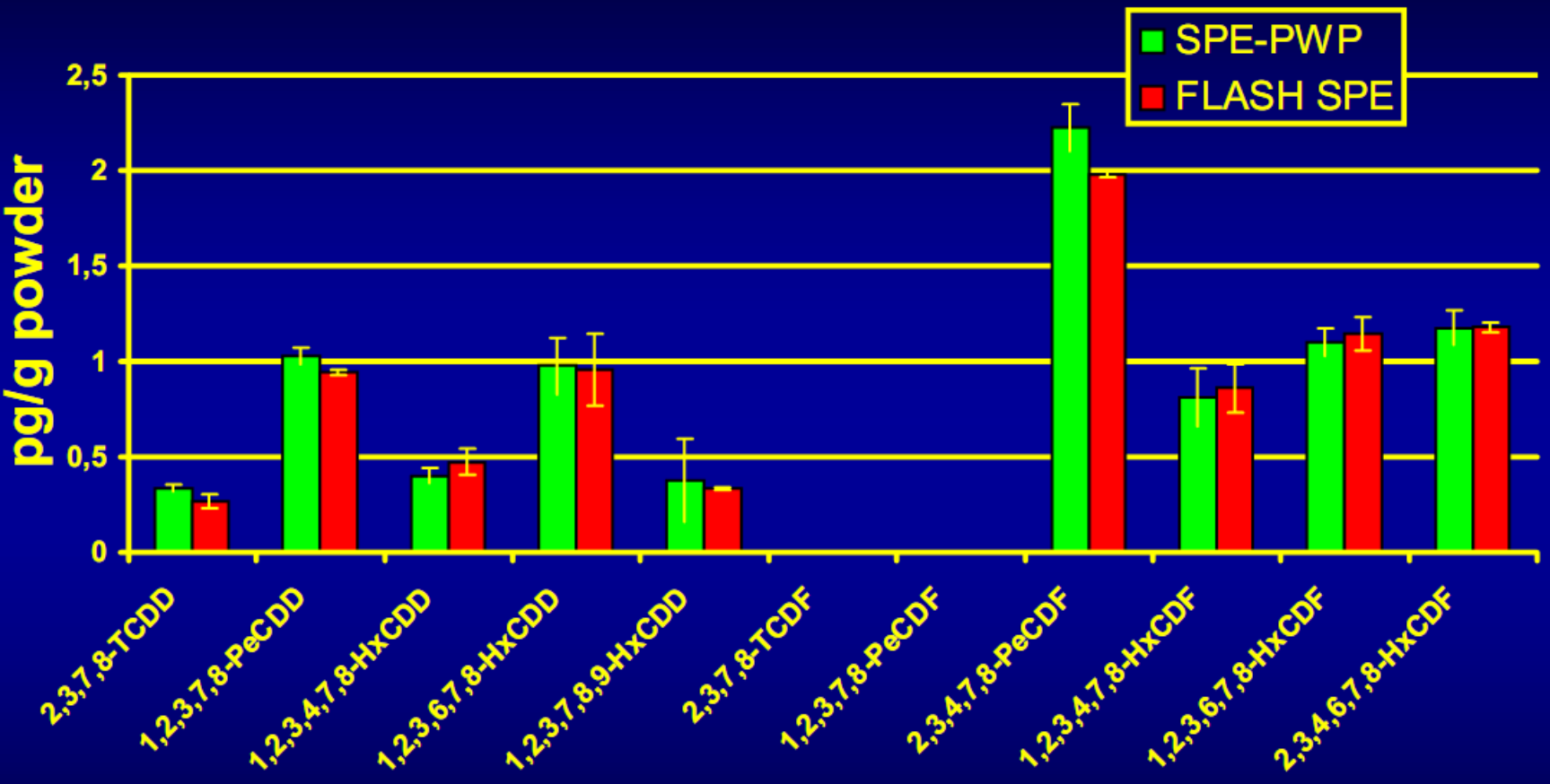
A process of extraction and cleaning passage – ways are practically without intervention from an operator, it eliminates influence of human factor on quality of results, and the use of ready – made columns reduce time of cleaning and helps the increase of degree of reproducibility of results.

Manual vs Automated



~ 8 g reconstituted CRM 607

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For the concentration of eluates after chrtomatographic columns evaporation of organic solvents is used evaporated systems of Turbo Vap and Rapid Vap Evaporation System (USA). These table devices allow simultaneously quickly steam from 6 to 50 eluates to the permanent volume in a vacuum or with the nitric blowing out non-destructive or losses of analyts.

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TurboVap

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RapidVap

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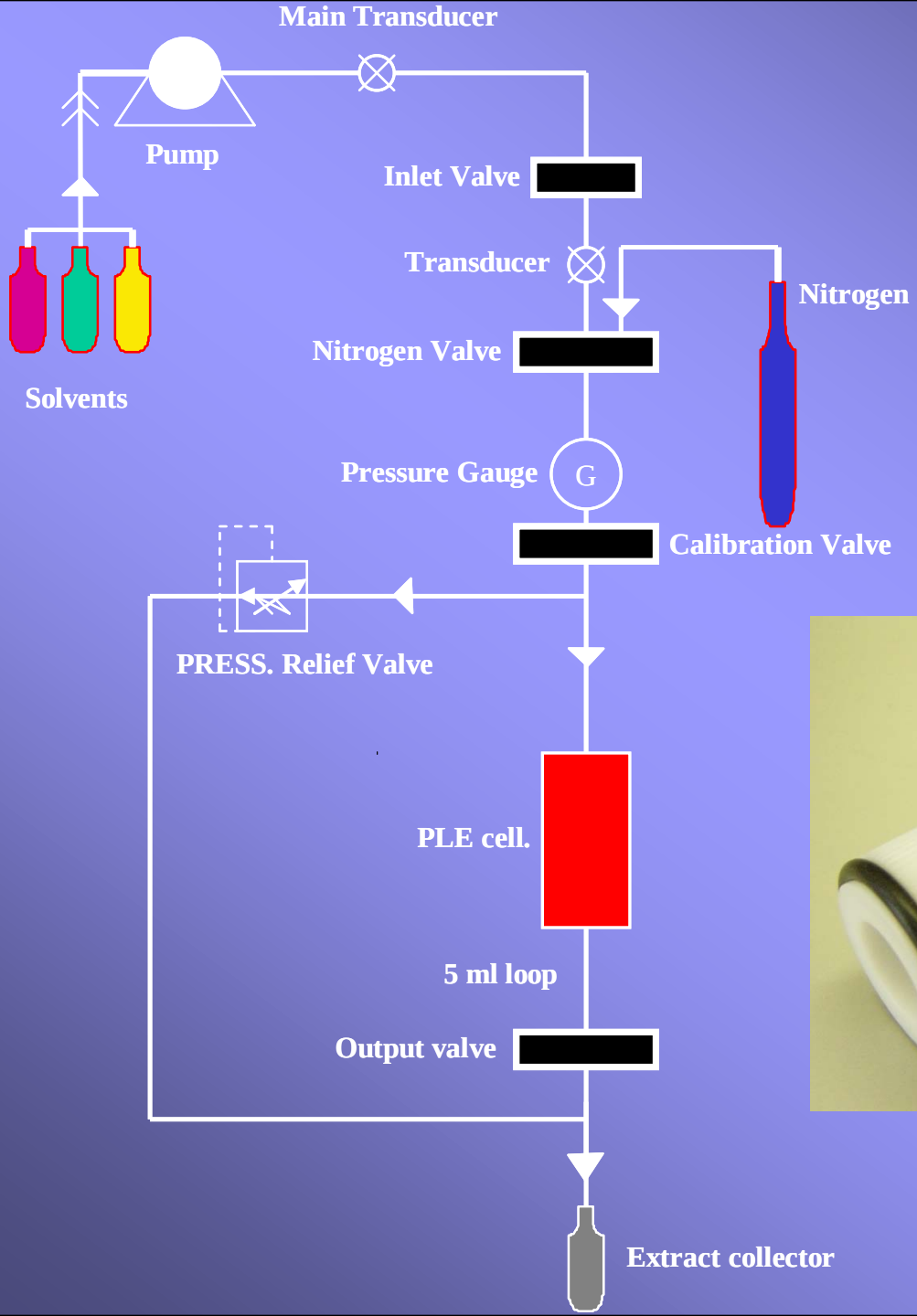
Dioxine Center of ECOHYNTOX

Recovery for PCDD/PCDF

Matrix	Extraction	Weight of sample, g	Recovery,%
Soya oil	-	2,5	78-115
Sunflower oil	-	2,5	80-110
Meat (pork)	PLE	5,0	70-107
Liver (pig)	PLE	10,0	75-101
Fish (salmon)	PLE	10,0	74-108
Sunflower cake	PLE	10,0	76-105
Corn	PLE	10,0	80-102
Soya	PLE	10,0	85-103

Chart of operations of the system of automatic extraction of PLE, FMS, USA



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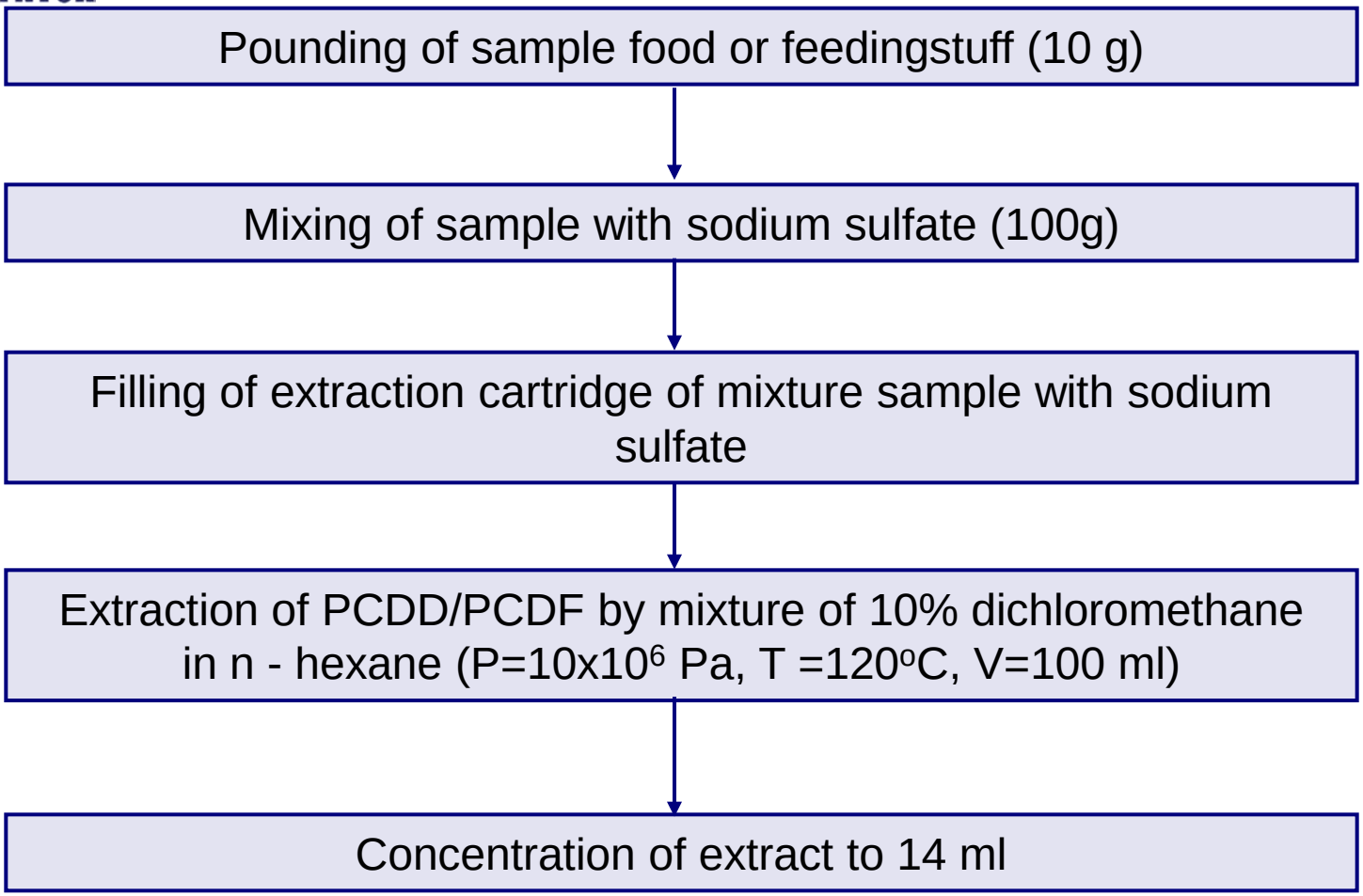
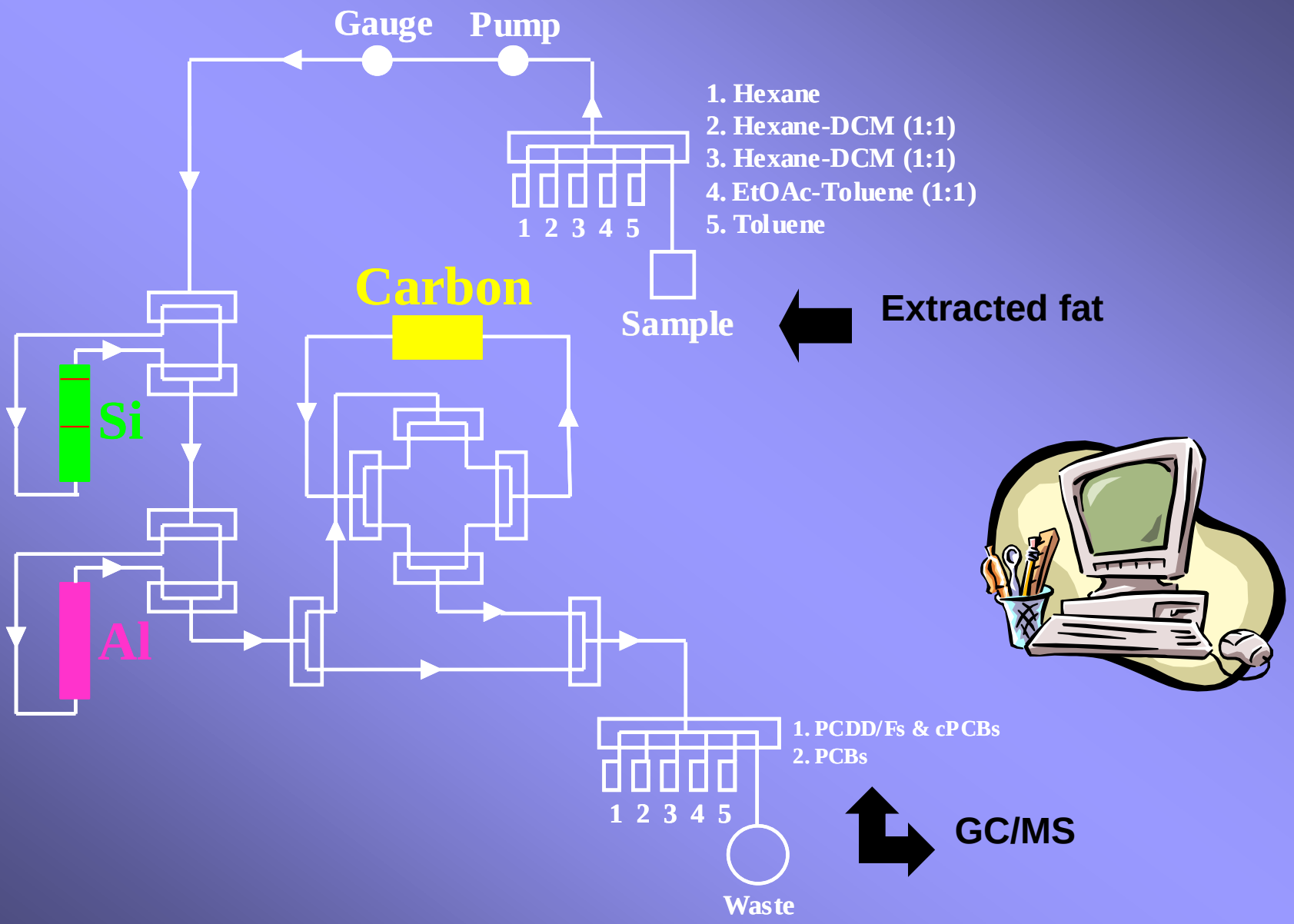


Sequence of operations of procedure of automatic extraction

Chart of the system for the automatic cleaning of extracts on Power-Prep™, FMS, USA



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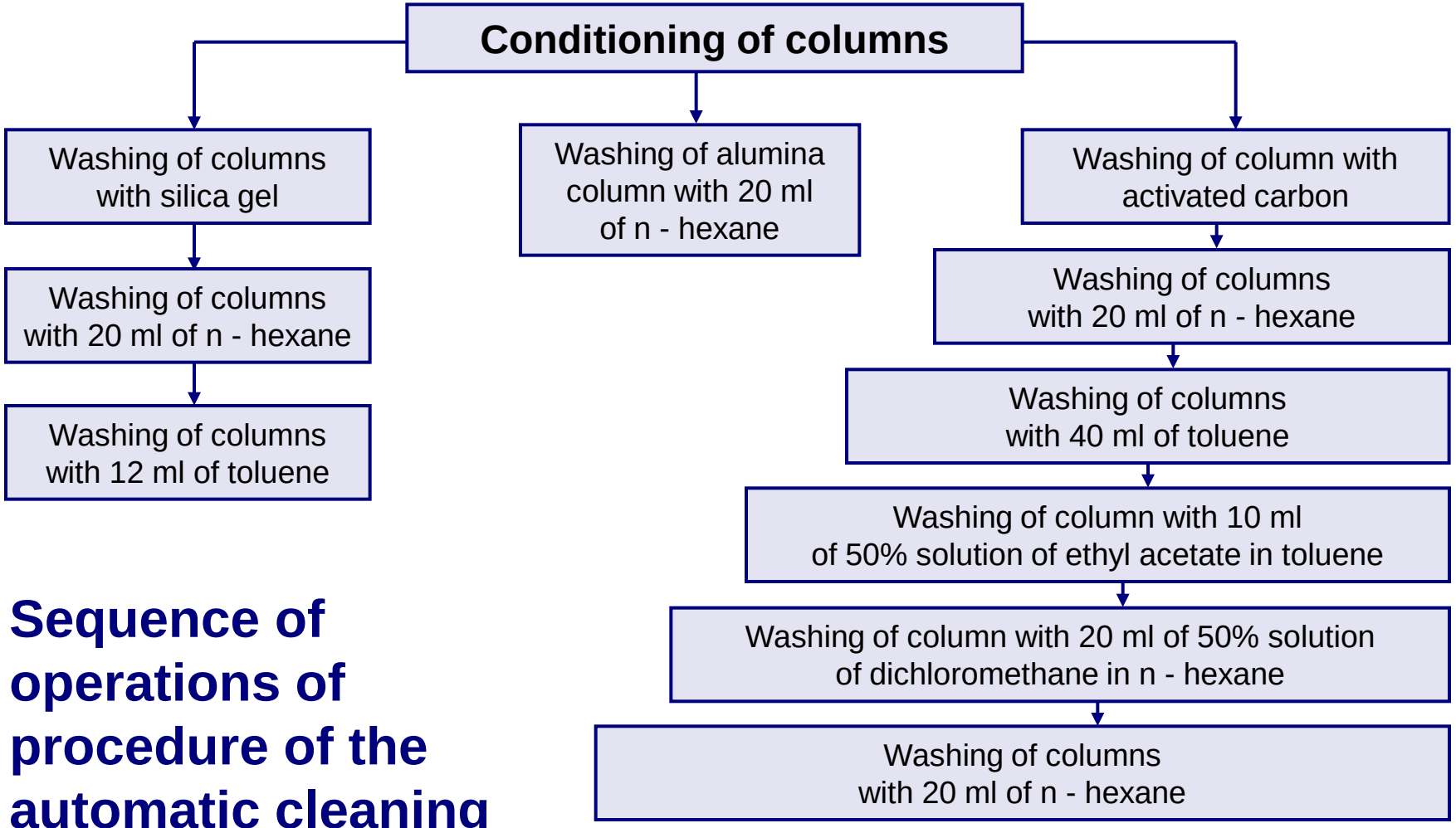

Sequence of operations of procedure of the automatic cleaning of extracts

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Sample Extract in 14 ml n-hexane

↓

Bring of sample extract on high capacity disposable silica column (HCDS)

↓

Elution of analyts from HCDS column in multilayer silica column by n-hexane (200ml)

↓

Elution of analyts from MLS column in alumina column aluminium of by n-hexane (90 ml)

↓

Washing of alumina column 2% solution dichloromethane in n-hexane (60 ml)

↓

Elution of analyts from alumina column in carbon column 50% solution dichloromethane in n-hexane (120 ml)

↓

Washing of carbon column 50% solution ethyl acetate in toluene (40 ml)

↓

Elution of analyts from carbon column by toluene (75 ml)

↓

Collection of effluent for concentration

Sequence of operations of procedure of the automatic cleaning of extracts

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The extract of sample must be ready before cleaning Program will be started. An extract is concentrated to 200 µl and then to 12 ml add a hexane. If extract of sample already in a hexane, it is not needed to concentrate an extract to 200 µl, and it is necessary to lead to the volume of extract to 12 ml.

Preliminary columns with silica gel, alumina and coal wash and air-condition in the system organic solvents (hexane, toluene, mixtures ethyl-acetate and toluene, dichloromethane and a hexane) for removal of fat.

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A sample must be inflicted on a column with silica gel of high capacity not less than, than 30 minutes prior to the start of cleaning Program. There is destruction of lipids of sample on this column, where upon elution a sample a hexane in the second silica gel column. Then dioxins elution from a column with silica gel in a column with the alumina by 2% solution of dichloromethane in a hexane. From a column with the alumina analyts elution 50% solution of dichloromethane in a hexane in a column with a carbon. From a column with the carbon dioxins elution toluene in retrograde. Then concentrate an eluate to 0,5 ml in the current of nitrogen with heating, 3 ml of nonane add, bring in internal standards (1,2,3,4 -[13C12]-TCDD and 1,2,3,7,8,9 - [13C12]-HCDD) and immediately beforet of analysis concentrate a sample in the current of nitrogen with heating to ~20 µl.

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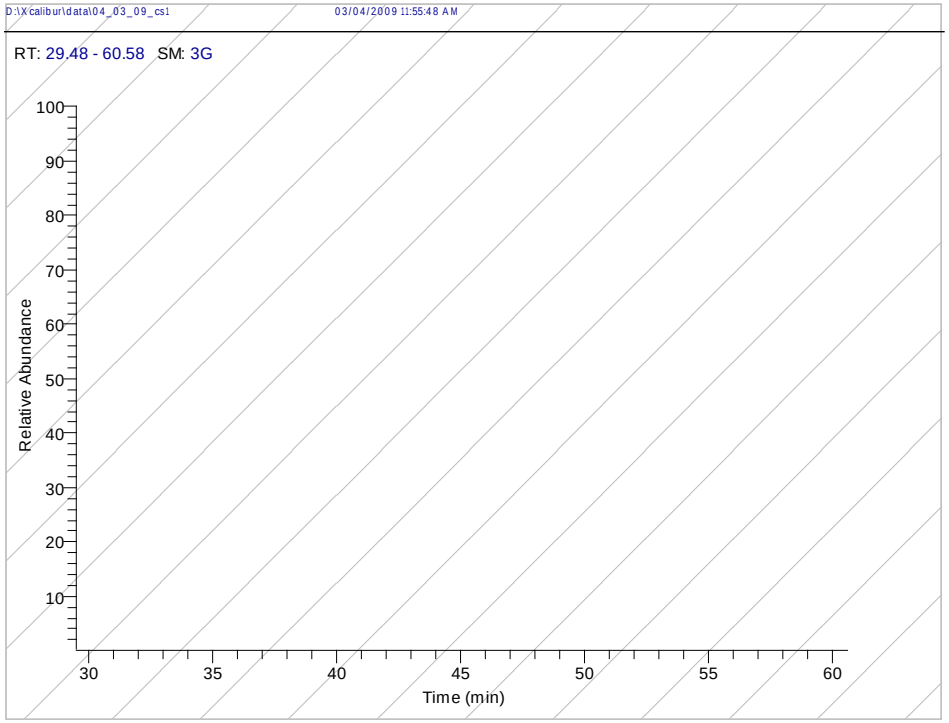
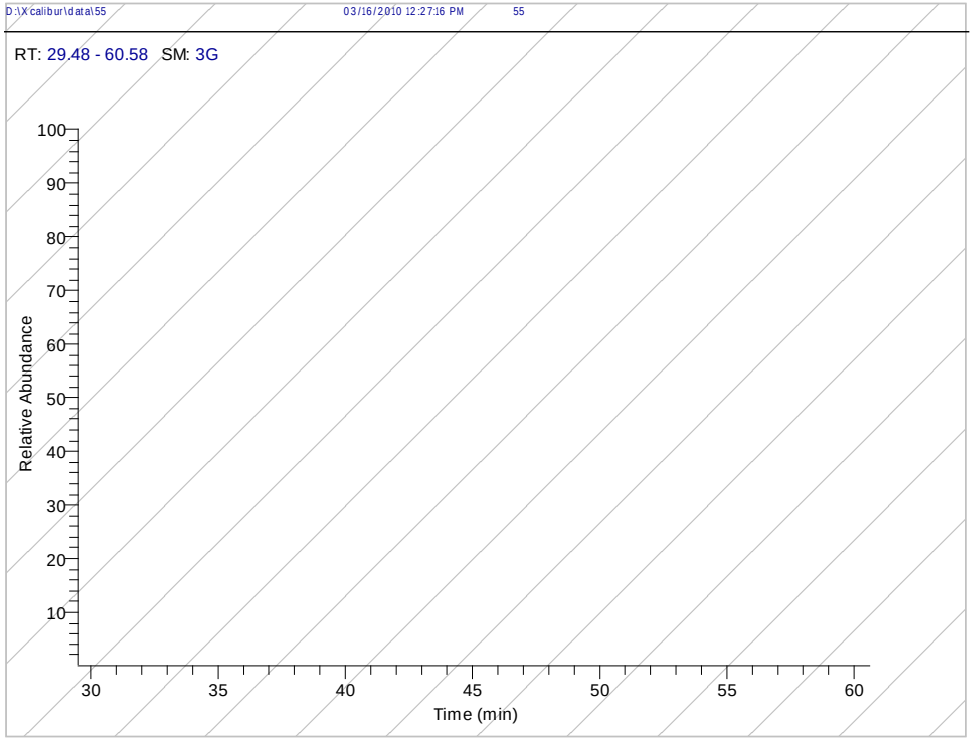
The cleaning program lasts approximately 90 minutes from start to finish. The last stage of elution of dioxins occupies 15 minutes.

Use of the systems of the automatic extraction and cleaning of extracts of FMS in Dioxine Center ECOHYNTOX showed that these systems are reliable and universal and allows to clear extracts from the different matrices of food, agricultural raw material, feedingstuff (meat, fish, vegetable oils, wheat, corn, sunflower cake and other) in accordance with norms of European Community. On next slides results of analysis of samples of different matrices are brought with the use of the systems FMS in Dioxine Center ECOHYNTOX.

Template for TCDD to OCDD
and TCDD to OCDF



Template for TCDD to OCDD and TCDD to OCDF



Template for TCDD to OCDD
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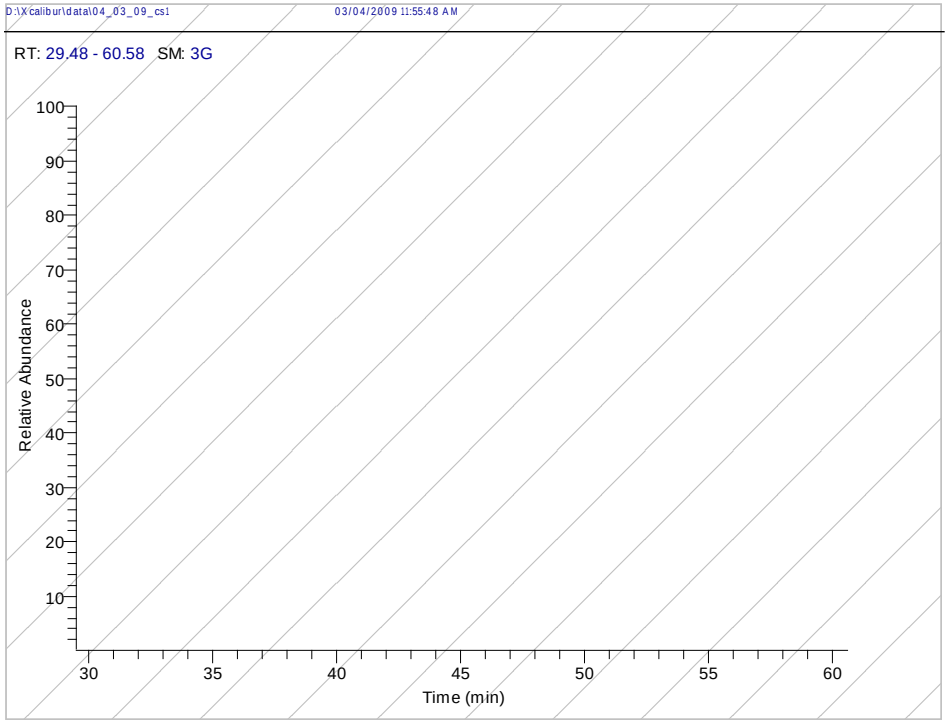

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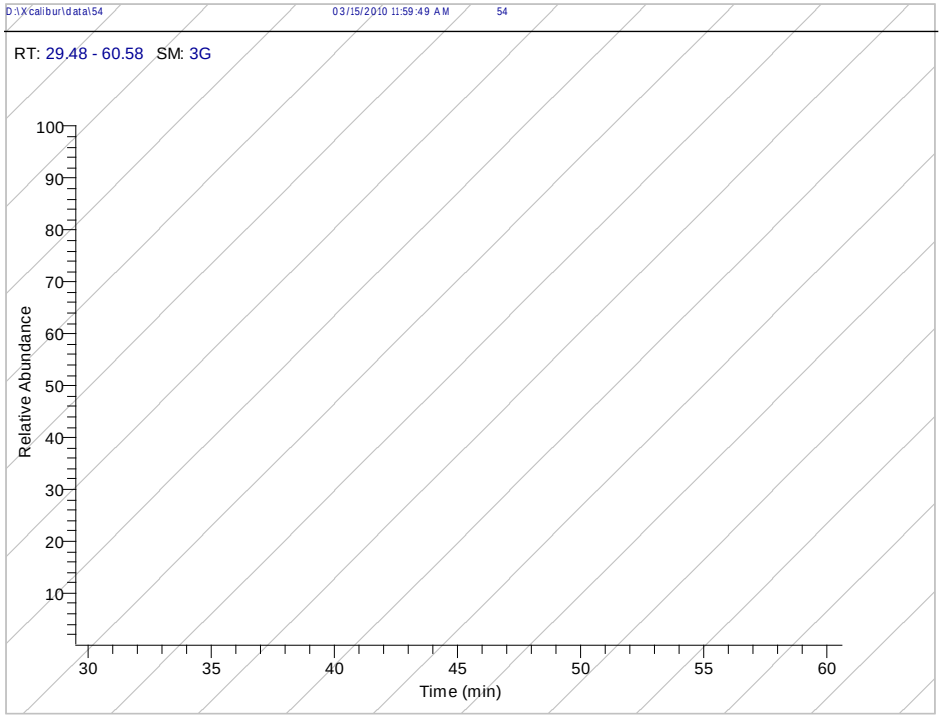

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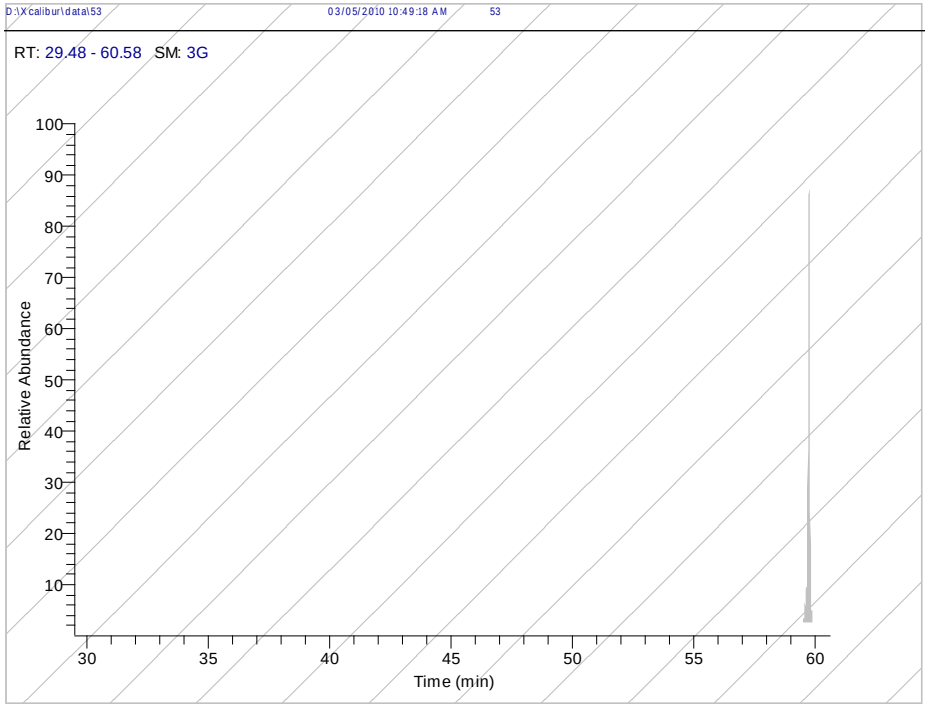

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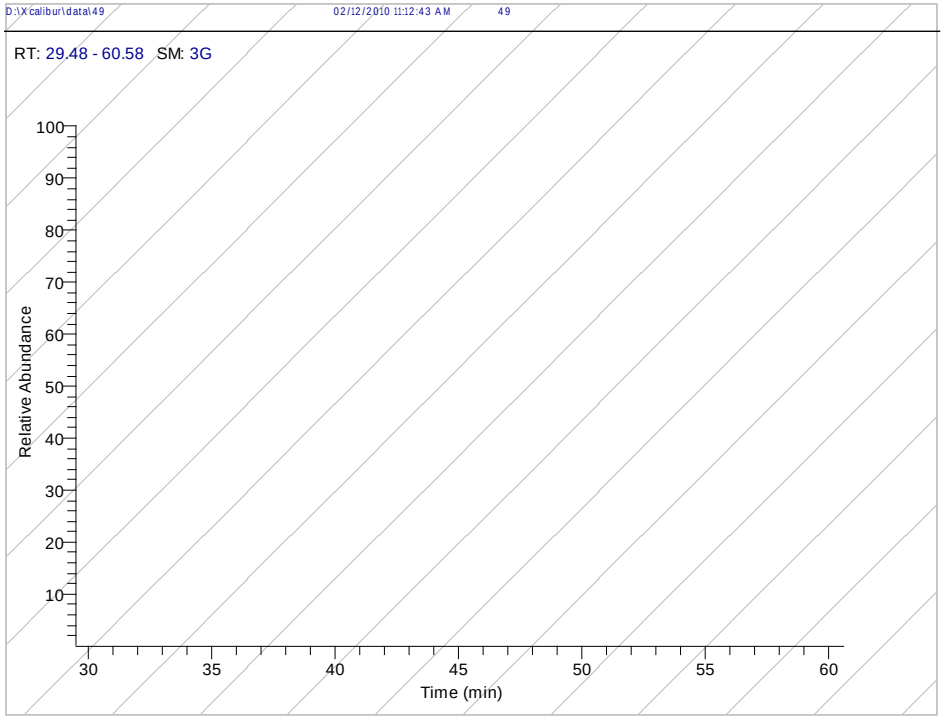
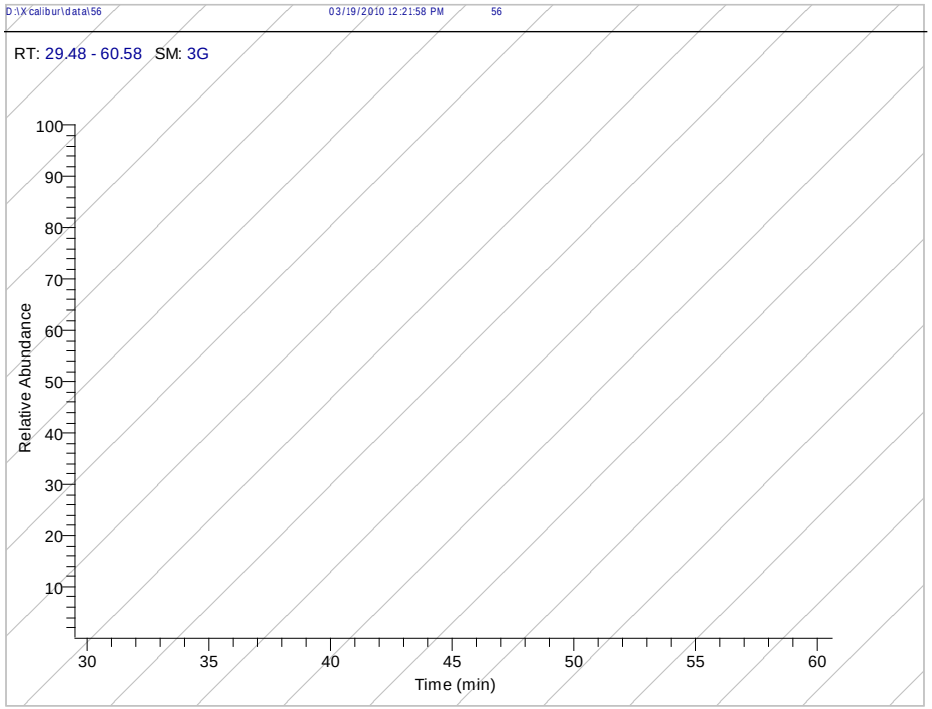
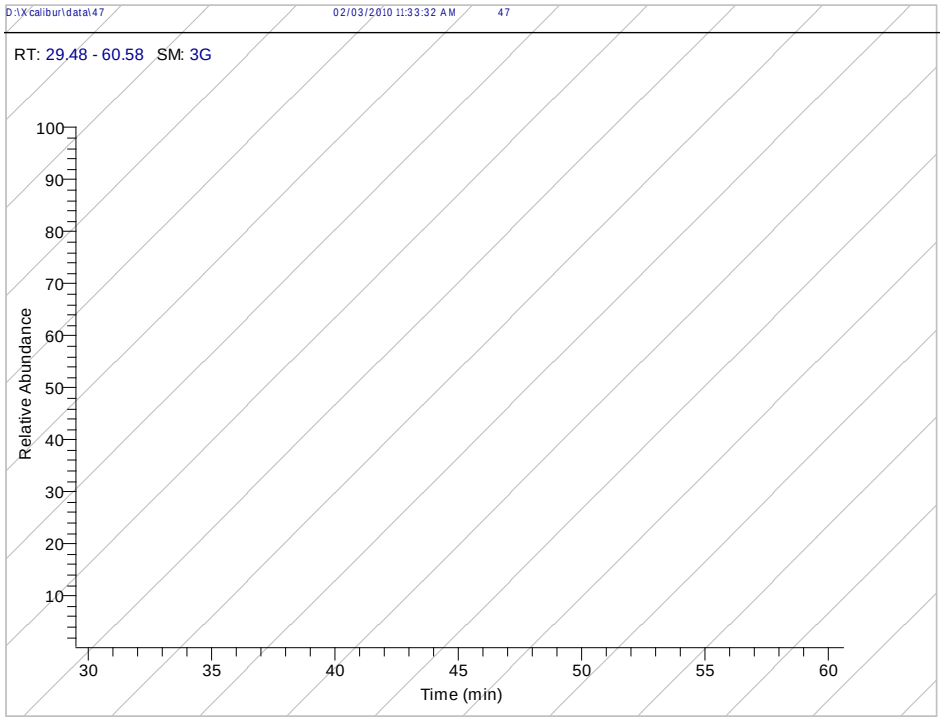


Template for TCDD to OCDD and TCDD to OCDF



Template for TCDD to OCDD and TCDD to OCDF



Template for TCDD to OCDD
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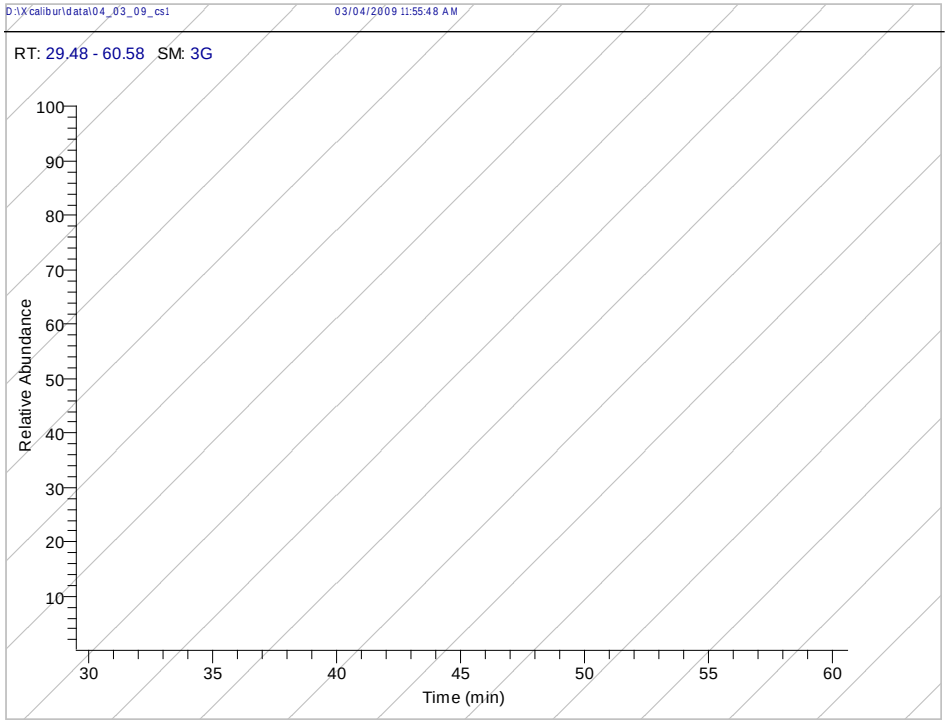

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As a result of analysis of four samples of feed - stuff corn which was supplied a private enterprise Dnepr - 2 (Kherson, Ukraine) in an address Meunerie Liegeois BV and Doens Food Ingredients BV (Netherlands), conducted in Dioxin Center ECOHYNTOX, with the use of Power - Prep system in combination with PLE, it was succeeded to prove that there is not a dioxine in the Ukrainian corn. and she did not could is the source of contamination of forage which was produced by the Dutch enterprise of Forfarmes and analogical German enterprise Reudink. Later, results on determination of dioxines in the Ukrainian corn of Dioxine Center ECOHYNTOX were confirmed in International LaboratoriesTLR, Rotterdam, Netherlands.

CONCLUSION

Use of the systems of automatic extraction and cleaning of FMS in the Dioxin center of ECOHYNTOX allowed:

to conduct the preparation of samples of different matrices of food, agricultural raw material, feedingstuff and biological liquids of man, containing PCDDs and PCDFs;

considerably to increase the productivity of work of the Dioxin center at the analysis of these xenobiotics;

to decrease the consumption of organic solvents;

to shorten time of teaching of personnel of analysts for implementation of operations of the stages of extraction and cleaning;

to promote a repeatability and quality of results of analysis due to diminishing of influence of human factor;

to prove that the Ukrainian corn is not the source of contamination of forage dioxins which produce Dutch and German enterprises.