Scientific Report
regarding the implementation of the project during the period December 16th, 2014 – December 15th, 2015

Project:	PN-II-ID-PCE-2011-3-0152
Project title:	Injection of large volumes of immiscible solvents with the mobile phase in high pressure liquid chromatography.
Contract number:	310/05.10.2011
Project Director:	Prof. Dr. Andrei Medvedovici
Time period for the report:	12.16.2014 –12.15.2015

Due to the rescheduling of the initial project for an extended period (until September 2016), this report covers the objectives and activities recorded in the project plan, Annex IV to the Grant Contract no. 310 / 05.10.2011.

The following objectives and activities are in discussion:

O1. Identify areas of application for the LVI of various immiscible solvents:
- ***O1A1.*** Applications in quality control of pharmaceuticals;
- ***O1A2.*** Applications in environmental quality control.

O2. Obtaining data regarding the process of large volumes injection for immiscible solvents in liquid chromatography:
- ***O2A1.*** Checking various organic components of the mobile phase (RP mechanism) that lends to LVI for immiscible diluents;
- ***O2A2.*** Checking the different separation mechanisms (NP, ANP, HILIC).

O3. Identification of potential application areas for LVI using various immiscible solvents:
- ***O3A1.*** Applications in food analysis.

O4. Possibilities for characterizing hydrophobicity of organic compounds by the injection of large volumes of diluents immiscible with the mobile phase - preliminary studies:
- ***O4A1.*** Study injection of large volumes of structured solvents in RPLC - preliminary studies.

O5. Dissemination of the results:
- ***O5A1.*** Publication of experimental data in ISI journals.
- ***O5A2.*** Participation in scientific conferences.

For O1A2 / O2A1:

The possibility of replacing acetonitrile with ethyl lactate in the composition of the mobile phase used for reverse phase liquid chromatography was evaluated. An environmental application was selected, referring to the separation of polynuclear aromatic hydrocarbons (PAHs) on a conventional stationary phase consisting in octadecyl chemically modified silica gel, considering the replacement of acetonitrile with ethyl lactate. The determination of the composition of the mobile phase containing ethyl lactate which produces both a retention and selectivity similar to those obtained with a mobile phase containing acetonitrile was targeted. Analytical application may be deemed feasible, though accepting some compromises in terms of order of elution, chromatographic efficiency and sensitivity (considering UV detection). Thermodynamic aspects regarding the chromatographic separation process for the two systems were also measured comparatively. The correlations between the order of elution and several molecular descriptors were equally be taken into consideration, illustrating the idea that the mechanism of separation is the same both for the use of ethyl lactate and acetonitrile.

The mixture subjected to separation was as following: naphthalene (A); acenaphthylene (B); fluorene (C); acenaphthene (D); phenanthrene (E); anthracene (F); fluoranthene (G); pyrene (H); chrysene (I); benzo(a)anthracene (J); benzo(b)fluoranthene (K); benzo(k)fluoranthene (L); benzo(a)pyrene (M); dibenzo(a, h)anthracene (N); benzo(g,h,i)perylene (A) and indeno (1,2,3-c,d)

pyrene (P).

The linear regression parameters, characterizing the linear relationship between the logarithm of the retention factor (log k) and the logarithm of the percentage of the organic modifier in the mobile phase are illustrated in the table below.

Table 1. The linear regression parameters for the log k = f (log% organic modifier) function.

Org. Modif.	Compound	A	B	C	D	E	F	G	H
Ethyl Lactate	Slope	-4.2411	-4.4725	-4.6544	-4.4837	-4.9476	-5.0278	-5.0658	-5.1404
	Intercept	7.7420	8.1768	8.7255	8.4442	9.2080	9.4031	9.5114	9.6595
	r_{xy}	0.9987	0.9987	0.9985	0.9984	0.9983	0.9985	0.9980	0.9984
ACN	Slope	-3.8469	-4.0150	-4.3572	-4.2085	-4.5166	-4.5389	-4.7739	-4.6100
	Intercept	7.4546	7.8538	8.6297	8.3726	8.9797	9.0741	9.5985	9.3603
	r_{xy}	0.9987	0.9999	0.9999	0.9987	0.9986	0.9999	0.9986	0.9999

Org. Modif.	Compound	I	J	K	L	M	N	O	P
Ethyl Lactate	Slope	-5.6073	-5.6707	-5.6837	-5.8652	-5.7701	-6.1766	-5.7674	-5.9837
	Intercept	10.6214	10.7593	10.8761	11.2247	11.0470	11.9068	11.1102	11.5444
	r_{xy}	0.9980	0.9981	0.9979	0.9976	0.9979	0.9968	0.9976	0.9977
ACN	Slope	-5.1810	-5.1667	-5.4330	-5.5098	-5.2363	-5.8086	-5.3121	-5.5983
	Intercept	10.5372	10.5335	11.1768	11.3432	10.8822	12.0437	11.1922	11.7115
	r_{xy}	0.9988	0.9999	0.9990	0.9990	0.9999	0.9999	0.9999	0.9993

The equivalence between the percentage of ethyl lactate and acetonitrile in the mobile phase, to provide a similar chromatographic retention is illustrated in Table 2.

Table 2. Mobile phase compositions containing ethyl lactate and acetonitrile resulting in a similar chromatographic retention of analytes of interest.

Analytes	**% Ethyl Lactate**				
% ACN	**70**	**65**	**62.5**	**60**	**55**
A	55.1	51.5	49.7	47.9	44.3
B	53.5	50.1	48.3	46.6	43.1
C	56.0	52.2	50.3	48.4	44.7
D	56.0	52.2	50.3	48.4	44.6
E	53.8	50.2	48.5	46.7	43.1
F	53.8	50.4	48.6	46.8	43.3
G	52.7	49.1	47.3	45.6	42.0
H	51.6	48.3	46.6	45.0	41.6
I	52.5	49.0	47.2	45.5	42.0
J	52.6	49.2	47.4	45.7	42.2
K	51.4	47.9	46.1	44.3	40.8
L	51.7	48.2	46.4	44.7	41.2
M	50.5	47.2	45.5	43.9	40.5
N	51.6	48.2	46.4	44.7	41.2
O	48.4	45.2	43.6	42.0	38.8
P	49.9	46.6	44.9	43.2	39.8
Average	***52.6***	***49.1***	***47.3***	***45.6***	***42.1***
SD	***2.10***	***1.96***	***1.90***	***1.83***	***1.70***

Mathematical relation $\%\,\text{Ethyl lactate} = 0.7 \times \%\,\text{Acetonitrile} + 3.6$ describes the right experimental conditions for which the chromatographic retention of analytes of interest is almost equal.

Collection of experimental data dependencies of the van't Hoff plot (ln x = f (1/T)) allowed the determination of the free enthalpy and entropy values associated to the chromatographic retention, when using acetonitrile/water or ethyl lactate/water mobile phases (see Table 3).

Functional plot between $-\Delta H^0$ and ln $k_{(T=30\ ^oC)}$, shown in Figure 1 b, illustrates that the compensation phenomenon of enthalpy by entropy obtained for the PAHs separation in both acetonitrile/water and ethyl lactate/water elution systems (Figure 1a), pleading for the preservation of the separation mechanism in the two elution conditions, is not due to experimental errors occurring during the determination of the enthalpy (in Figures 1a and b, the black circles correspond to analytes eluted in the ethyl lactate/water system, while gray circles correspond to analytes eluted in the acetonitrile/water system).

Table 3. Thermodynamic aspects illustrating retention of the PAHs when using mobile phases with ethyl lactate or acetonitrile (situation corresponding to a similar selectivity).

Analyte	Acetonitrile/water = 70/30 (v/v)					
	B	A	r_{xy}	ΔH^0 $KJmol^{-1}K^{-1}$	ΔS^0 $Jmol^{-1}K^{-1}$	ΔG^0 (25 °C) $KJmol^{-1}K^{-1}$
A	878	-2.21	0.9847	-7.30	-7.6	-5.04
B	1043	-2.55	0.9923	-8.67	-10.4	-5.56
C	1108	-2.43	0.9922	-9.21	-9.5	-6.39
D	1025	-2.11	0.9860	-8.52	-6.7	-6.51
E	1138	-2.40	0.9876	-9.46	-9.2	-6.73
F	1276	-2.74	0.9926	-10.61	-12.0	-7.03
G	1290	-2.57	0.9886	-10.73	-10.6	-7.57
H	1422	-2.87	0.9922	-11.83	-13.1	-7.92
I	1538	-2.96	0.9906	-12.79	-13.9	-8.65
J	1594	-3.11	0.9929	-13.25	-15.1	-8.76
K	1741	-3.24	0.9915	-14.48	-16.2	-9.65
L	1801	-3.38	0.9919	-14.98	-17.4	-9.80
M	1899	-3.62	0.9931	-15.79	-19.3	-10.02
N	2073	-3.81	0.9930	-17.24	-20.9	-11.00
O	2024	-3.80	0.9937	-16.83	-20.8	-10.62
P	2072	-3.82	0.9925	-17.23	-21.0	-10.97
Analyte	**Ethyl Lactate/water = 45/55 (v/v)**					
	B	**A**	r_{xy}	ΔH^0 $KJmol^{-1}K^{-1}$	ΔS^0 $Jmol^{-1}K^{-1}$	ΔG^0 (25 °C) $KJmol^{-1}K^{-1}$
A	1427	-2.97	0.9889	-11.86	-13.9	-7.71
B	1495	-3.08	0.9913	-12.43	-14.9	-8.00
C	1765	-3.43	0.9927	-14.67	-17.8	-9.37
D	1739	-3.34	0.9922	-14.46	-17.0	-9.39
E	1780	-3.49	0.9924	-14.80	-18.3	-9.34
F	1876	-3.67	0.9919	-15.60	-19.8	-9.70
G	1921	-3.71	0.9913	-15.97	-20.1	-9.99
H	1925	-3.68	0.9913	-16.00	-19.8	-10.09
I	2283	-4.44	0.9897	-18.98	-26.2	-11.18
J	2281	-4.36	0.9900	-18.97	-25.5	-11.36
K	2466	-4.74	0.9894	-20.51	-28.6	-11.97
L	2573	-5.00	0.9892	-21.39	-30.8	-12.22
M	2514	-4.86	0.9893	-20.90	-29.7	-12.06
N	2857	-5.54	0.9887	-23.75	-35.3	-13.23
O	2578	-4.91	0.9891	-21.43	-30.1	-12.47
P	2774	-5.40	0.9890	-23.06	-34.1	-12.89

The correlations established between retention data for analytes of interest in the two alternative elution systems and molecular descriptors associated with these analytes, as described in Table 4 pleads equally for the fact that the separation mechanism is not changing when switching from acetonitrile to ethyl lactate as the organic modifier in the mobile phase.

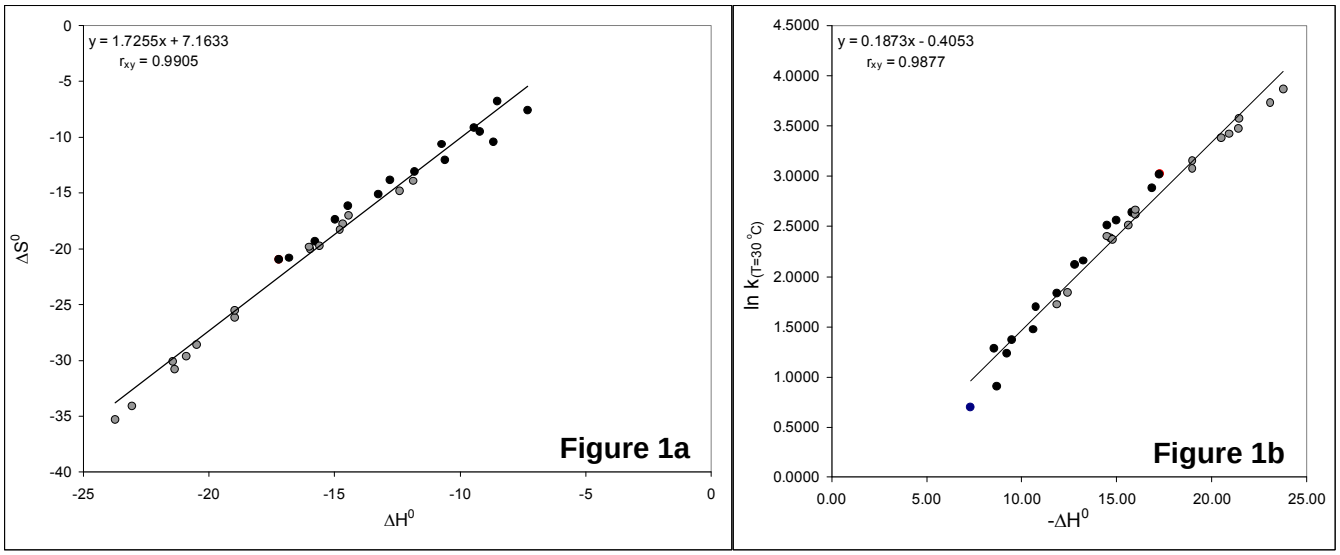


Figure 1. Enthalpy/entropy compensation observed for the separation of PAHs on an ODS stationary phase when using acetonitrile/water or ethyl lactate/water elution systems.

Table 4. Relationships between retention data obtained in the two alternative elution systems and the molecular descriptors of the target analytes.

Organic modifier	Descriptor	M_w	V_w	log P	S	□	F	L/B
	Function	M_w=f(log k)	V_w=f(log k)	Log P=f(log k)	Log S=f(log k)	χ=f(log k)	F=f(log k)	L/B=f(log k)
ACN	Average r_{xy}	0.9941	0.9918	0.9937	-0.9701	0.9964	0.9974	0.3540
	SD	0.0010	0.0017	0.0009	0.0007	0.0006	0.0010	0.0111
Ethyl Lactate	Average r_{xy}	0.9800	0.9852	0.9865	-0.9584	0.9832	0.9874	0.4367
	SD	0.0040	0.0043	0.0024	0.0048	0.0021	0.0023	0.0156

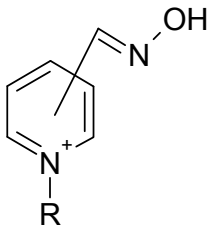
Conclusions:

Replacement of acetonitrile by ethyl lactate in mobile phases for RPLC appears to be feasible. This may be considered an useful issue in the actual general tendency of greening chromatographic processes. Ethyl lactate is fully miscible with water and produces pressure drop regimes accepted by the usual available instrumentation existing on the market. For the tested solutes, similar retention was obtained in mobile phases containing about 15% less ethyl lactate compared to acetonitrile based ones. However, for a similar selectivity of the separation process, around 20-25% less ethyl lactate compared to the acetonitrile elution conditions is needed. Although optimized selectivity with ethyl lactate in the mobile phase does not differ substantially from that obtained under acetonitrile elution conditions, the required duration of the separation process is larger. As observed from the van Deemter plot, the increased resistance to the mass transfer in the mobile phase, when using ethyl lactate, imposes working conditions close to the optimal speed of the eluent through the column, further increase of the flow rate generating an important reduction of efficiency. Two major drawbacks make acetonitrile replacement by ethyl lactate to be considered with precautions. The first one refers to detection reasons: the cut-off wavelength of ethyl lactate is significantly much higher than for acetonitrile. Consequently, when using UV detection, especially for rich organic modifier mobile phase compositions and for separation of solutes lacking of chromophores, detectability under ethyl lactate elution conditions may be fully compromised. The second drawback refers to the lack of availability (at least for the moment) on the market of chromatographic grade ethyl lactate. To conclude, acetonitrile replacement by ethyl lactate in mobile phases used in liquid chromatography is possible, but should be considered with precaution.

For O1A1 / O2A2:

The possibility of using a stationary phase allowing multimodal separation regime (RP + HILIC) for the evaluation of the intrinsic lipophilicity of a family of solutes (quaternary oximes), which are used as reactivators of organophosphorus inhibited acetylcholinesterase (compounds that can be potentially used as antidotes against poisoning with organo-phosphorous derivatives) was evaluated.

The considered set of compounds is structurally illustrated in the Figure 2.



#	Substituent (R)	Substituent (R)	Oximic function position	Acronym
1	Ethyl	$-C_2H_5$	2, 3, 4	2-PAE, 3-PAE, 4-PAE
2	Buthyl	$-C_4H_9$	2, 3	2-PAB, 3-PAB
3	Hexyl	$-C_6H_{13}$	2, 3, 4	2-PAH, 3-PAH, 4-PAH
4	Octyl	$-C_8H_{17}$	2, 3, 4	2-PAO, 3-PAO, 4-PAO
5	Decyl	$-C_{10}H_{21}$	2, 3	2-PAD, 3-PAD
6	Dodecyl	$-C_{12}H_{25}$	2, 3, 4	2-PAL, 3-PAL, 4-PAL
7	Benzyl	$-CH_2-C_6H_5$	2, 3, 4	2-PABn, 3-PABn, 4-PABn
8	Ethyl-phenyl	$-(CH_2)_2-C_6H_5$	2, 3, 4	2-PAPE, 3-PAPE, 4-PAPE
9	Propyl-phenyl	$-(CH_2)_3-C_6H_5$	3	3-PAPP
10	Butyl-phenyl	$-(CH_2)_4-C_6H_5$	3, 4	3-PAPB, 4-PAPB
11	4-Methylbenzyl	$-CH_2-C_6H_4-CH_3$	2, 3, 4	2-PAMB, 3-PAMB, 4-PAMB
12	4-*t*-Butylbenzyl	$-CH_2-C_6H_4-C(CH_3)_3$	3, 4	3-PATB, 4-PATB

Figure 2. The chemical structure of the investigated analytes.

The stationary phase was a chemically dodecyl-diol modified silica gel. The interaction of analytes with the hydrocarbon chain determines a reversed phase separation mechanism. However, when using an organic modifier rich (i.e. acetonitrile) mobile phase, a water layer held by hydrogen bonds at the level of the terminal hydroxyl groups is obtained, and the stationary phase may operate in the HILIC mode. Due to the simultaneous occurrence of both separation mechanisms, depending on the composition of the mobile phase, the chromatographic retention is increased at the two extremes of the interval in which the percentage of the organic modifier in the mobile phase may be varied. In the middle range, low retention is expected, generated only through the mutual transfer of the analyte between the lipophilic layer associated to the hydrocarbon chain and a hydrophilic layer of water retained by the terminal hydroxyl groups. The process is illustrated in Figure 3.

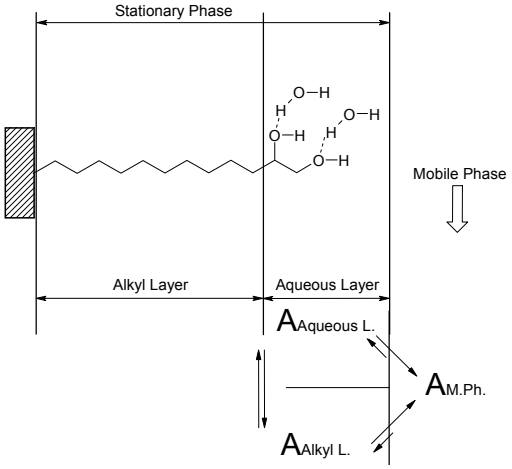


Figure 3. Possible interactions of the analyte with a stationary phase operating in a bimodal way.

In the above illustrated context, the retention of analytes in the stationary phase through using mobile phases with an organic modifier content ranging between 0 - 100%, will follow a parabolic profile. Minimum retention coincides with the situation illustrated in Figure 3. In such a case, the analyte retention will univocally depend only on the mutual transfer between the lipophilic and hydrophilic layers, and may be accepted as a descriptor of the intrinsic lipophilicity, and therefore, may be correlated log P.

The parabolic retention profiles obtained on this stationary phase ("U" shaped retention profiles) may generate information that, at their turn, may be correlated with log P values of the solutes. Starting from the primary plots k=f(% organic modifier) or log k=f(%organic modifier), a series of retention descriptors correlated to the lipophilicity of the solutes may result, as it follows: k_{min} and log k_{min}, representing the minimum retention factor (or its logarithm), as it results from the retention profile; ISOELUT and LOGISOELUT, representing the % of the organic modifier (or its logarithm) in the mobile phase corresponding to the lowest chromatographic retention, as it results from the application of the minimum condition to the binomial function best fitting the retention profile; ISOELUT1 and LOGISOELUT1 representing the % of the organic modifier (or its logarithm) in the mobile phase corresponding to the lowest chromatographic retention, as it results from the equality condition applied to the linear functions describing the decreasing and the increasing sides of the retention profile; ISOELUT2 and LOGISOELUT2 representing the % of the organic modifier (or its logarithm) in the mobile phase corresponding to the lowest chromatographic retention, as it results from the equality condition applied to the power functions describing the decreasing and the increasing sides of the retention profile; k_w^{lin} and log k_w^{lin}, representing the extrapolation to zero of the linear relationship between k or log k and the percentage of the organic modifier in the mobile phase (this corresponds to the hypothetical situation of a mobile phase consisting purely of water); k_w^{bin} and log k_w^{bin}, representing the extrapolation to zero of the binomial relationship between k or log k and the percentage of the organic modifier in the mobile phase (this corresponds to the hypothetical situation of a mobile phase consisting purely of water); Hyl and log Hyl, representing the extrapolation to 100% organic modifier in the mobile phase, considering a linear functional relationship applied to experimental points obtained in the HILIC interval of the retention profile.

The above described situations are illustrated in Figure 4a and b.

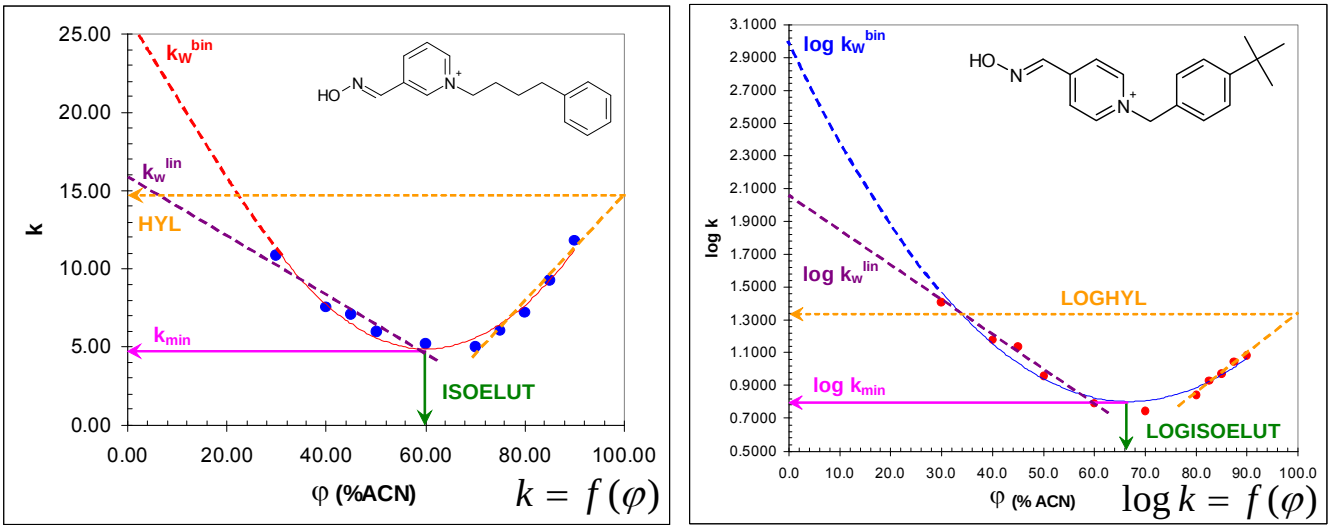


Figure 4. <Lipophilicity descriptors, as resulting from the retention profiles obtained for the studied solutes on the bimodal functioning stationary phase.

The values of the experimentally obtained lipophilicity descriptors were correlated to log P values computed via various mathematical models. Rezults are displayed in Table 5.

Conclusions:

One can conclude that the retention profile resulting from the chromatographic separation on a stationary phase that function in a bimodal way (RP + HILIC) may produce descriptors of the molecular lipophilicity highly correlated to log P values computed via widely accepted mathematical algorithms. Among these experimental descriptors, the minimum retention values (as such or the corresponding logarithm, k_{min} and log k_{min}) and the values of the percentages of the organic modifier in the mobile phase producing the lowest retention (ISOELUT or LOGISOELUT) are better correlated to

computed log P values resulting from chemometric approaches.

Table 5. Correlations between experimentally determined lipophilicity descriptors and log P values computed from different mathematical algorithms.

Correlation Coefficients											
Correlated data sets	ALOGPs	AC logP	miLogP	KOWWIN	XLOGP2	XLOGP3	Hy	MLOGP	ALOGP	LogD7	SlogD7.4
k_{min}	**0.958**	**0.977**	**0.934**	**0.963**	**0.977**	**0.983**	-0.758	**0.935**	**0.943**	***0.884***	**0.969**
log k_{min}	**0.938**	**0.977**	**0.927**	**0.973**	**0.964**	**0.978**	***-0.849***	**0.954**	**0.944**	**0.918**	**0.969**
ISOELUT	***0.842***	**0.915**	***0.835***	**0.942**	***0.895***	***0.899***	***-0.891***	**0.914**	**0.904**	**0.931**	**0.902**
LOG ISOELUT	***0.809***	***0.881***	***0.810***	**0.904**	***0.865***	***0.873***	***-0.874***	***0.880***	***0.855***	***0.894***	***0.864***
ISOELUT 1	0.479	0.512	0.460	0.508	0.484	0.529	-0.518	0.577	0.393	0.537	0.503
LOG ISOELUT 1	0.678	0.740	0.661	0.752	0.700	0.750	***-0.817***	***0.806***	0.640	0.788	0.738
ISOELUT 2	0.552	0.598	0.526	0.602	0.558	0.621	-0.730	0.668	0.493	0.642	0.609
LOG ISOELUT2	0.193	0.209	0.117	0.214	0.201	0.226	-0.267	0.240	0.135	0.232	0.227
k_w^{lin}	0.788	0.784	0.767	0.753	0.793	0.787	-0.546	***0.803***	0.770	0.652	0.770
log k_w^{lin}	***0.847***	***0.862***	***0.836***	***0.843***	***0.862***	***0.863***	-0.672	***0.878***	***0.840***	0.766	***0.845***
k_w^{bin}	0.754	***0.812***	0.756	0.787	***0.817***	***0.800***	-0.570	***0.814***	0.778	0.714	0.771
log k_w^{bin}	0.139	0.278	0.202	0.304	0.246	0.239	-0.377	0.257	0.259	0.374	0.230
HYL	0.488	0.472	0.479	0.413	0.458	0.502	-0.327	0.520	0.363	0.363	0.464
LOG HYL	0.430	0.405	0.420	0.346	0.400	0.440	-0.245	0.464	0.298	0.291	0.396

For O3A1:

The possibility of a holistic comparison by means of chemometric methods of complex mixtures of natural origins has been investigated, more precisely commercially available green teas. The application of the well known chemometric algorithms PCA and HCA was made to a series of data representing RPLC chromatograms (UV detection) or (+)/(-) mass spectrograms of the green teas extracts. An alternative method for the evaluation of the results, based on the linear regression principles is also proposed. Such alternative evaluation algorithms are useful due to the limited ability of the chemometric softwares to process very large sets of data (i.e. the Statistica software accepts correlation matrices with a maximum of 1000 variables, while chromatograms or MS spectra are data sets with more than 8000 items). As long as the chemometric techniques are based on a simple comparison of shapes, it seems evident that a higher frequency data acquisition rate will enable a more accurate reproduction of the experimental profiles). The way the instrumental variability (repeatability) affects the discrimination power of the chemometric methods was also investigated.

Five types of commercially available green teas (noted as T1 to T5) were extracted in ethanol (0.1 g of solid sample was mixed with 2 mL of ethanol, then vortexed 1 min at 2500 rpm, and next, kept in dark for 7 days; centrifugation and isolation of the supernatant ended the sample preparation procedure). The ethanol extracts were separated through reversed phase liquid chromatography with UV detection, on an Eclipse XDB C18 column (15 cm L × 4.6 mm i.d. × 3.5 µm d.p), with a mobile phase containing acetonitrile and aqueous 0.1% formic acid solution (v/v), eluted in a gradient composition profile as it follows: Time (min)/ % of organic modifier (v/v): 0.0/5%; 5.0/10%; 20.0/35%; 25.0/55%; 30.0/100%; 35.0/100%; 35.01/5%; 45.0/5%. Data acquisition frequency was 5 Hz. The duration of the chromatographic separation was 35 min, and consequently, the data set illustrating a chromatogram contains 10500 items. Each tea extract was injected five times repeatedly. For PCA and HCA, the data sets were reduced through averaging each 10 consecutive items. Mass spectra were obtained through the direct infusion of the ethanol extracts in the electrospray ion source of a mass spectrometer, using a flow made from acetonitrile and 0.1% aqueous solution of formic acid =

1/1 (v/v) and a rate of 0.5 mL/min. Mass spectra resulting from monitoring of positive and negative ions were measured with a spectral resolution of 0.1 a.m.u., ranging from 20 to 1100 a.m.u. The resulting data sets contained 10800 items. For PCA and HCA, the data sets were reduced through averaging each 10 consecutive items.

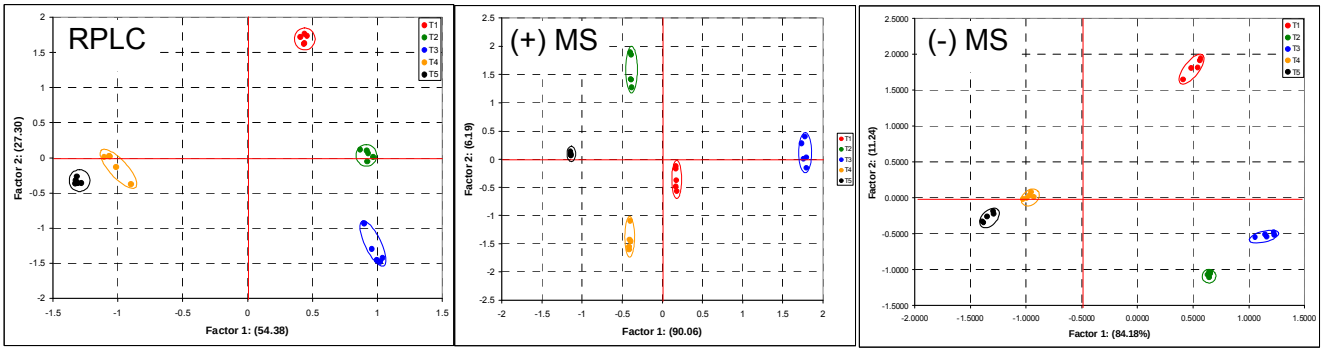


Figure 5. PCA representation of data produced through RPLC-UV, (+) and (-) MS investigation of the five types of green teas.

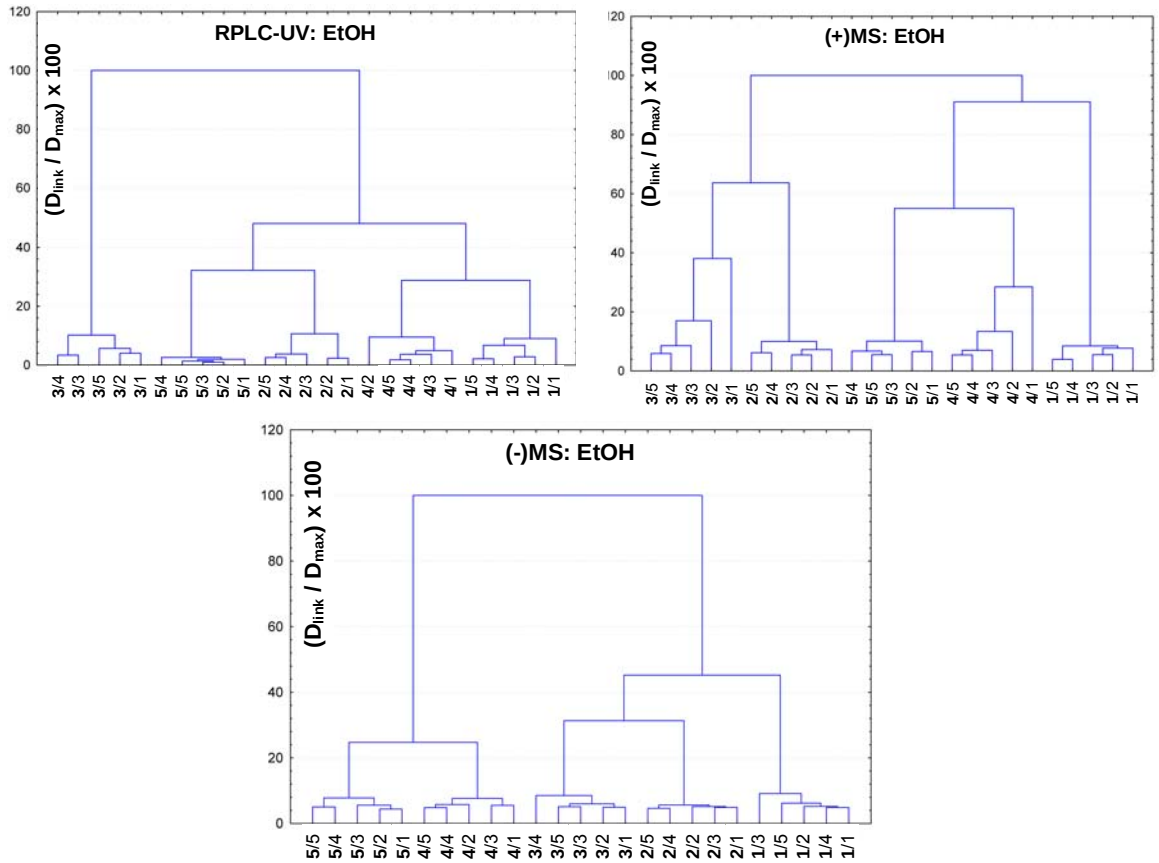


Figure 6. HCA representation of data produced through RPLC-UV, (+) and (-) MS investigation of the five types of green teas.

The results of the comparison through PCA of the green tea types (chromatograms, (+) MS and (-)MS spectra, respectively) are illustrated in Figure 5 a-c. One can observe that PCA discriminate against the five types of teas. One can also assume that the instrumental variability does not affect the discrimination power of the PCA technique.

The results of the comparison through HCA of the green tea types (chromatograms, (+) MS and (-)MS spectra, respectively) are illustrated in Figure 6 a-c. One can observe that HCA discriminate

against the five types of teas. One can also assume that the instrumental variability does not affect the discrimination power of the HCA technique. HCA applied to chromatograms associates T1 and T4, and T2 and T5 respectively. HCA applied to (+) mass spectra associates T2 and T3, and T4 and T5 respectively. HCA applied to (-) mass spectra associates T2 and T3, and T4 and T5 respectively.

A new approach refers to the comparison of the experimental data sets by means of the linear regression. The 5 data sets resulting for each tea (T_{x1} - T_{x5}) are compared through the consecutive representation of two data sets (one placed on the Ox and the other on the Oy axes, as illustrated in Figure 7). For such a functional relationship, one can calculate the parameters of the linear regression (more precisely the slope, the intercept and the coefficient of correlation). From the 20 possible arrangements (arrangements of 5 data sets taken as 2), one can calculate the means and standard deviations associated to the slopes, intercepts and correlation coefficients. For each of the linear regression parameters it is possible to approximate a normal variation interval (means +/- 2 x standard deviation).

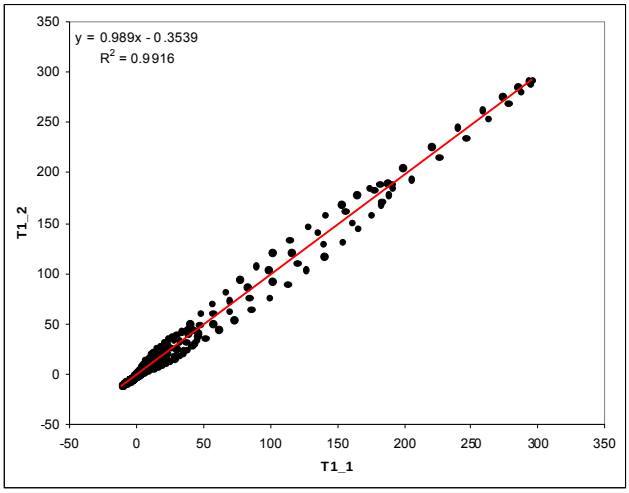


Figure 7. Comparison through the linear regression of the two data sets resulting from the consecutive RPLC analysis of an ethanol extract of a green tea sample.

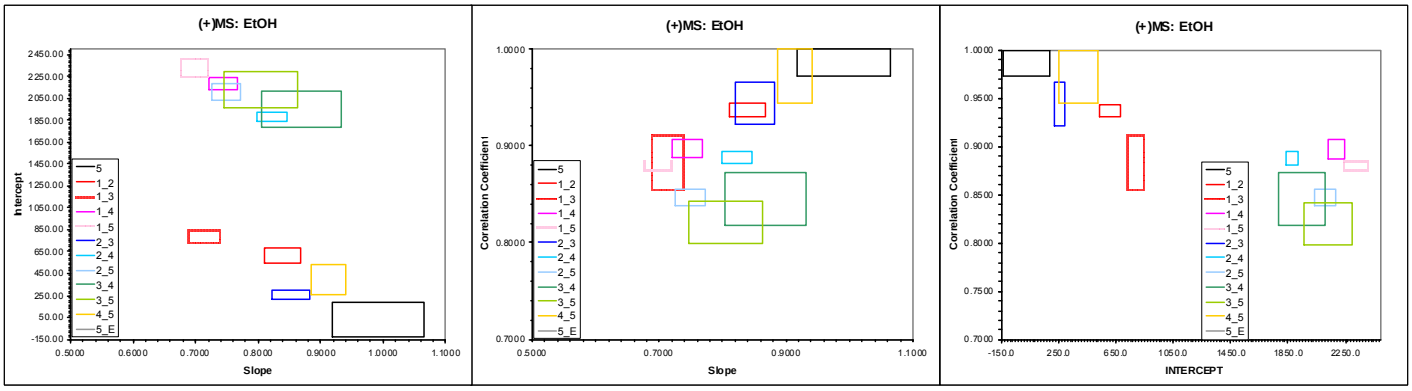


Figure 8. Projections of the volumes in the three planes of the Cartesian space resulting from comparison of the (+)MS spectra of the ethanol extracts from green teas, represented through the reduction of the dimensionality from 10800 variables to only 3).

When comparing 2 teas (for instance T_x and T_y) two sets of 5 different row data are compared. The comparison T_x/T_y will keep the rows associated to T_x in the Ox axes. 25 linear regression are thus obtained, characterized by means of the slopes, intercepts and correlation coefficients. It is possible to compute mean values, standard deviations and normal variation intervals. Tridimensional representation of the normal variation intervals of the three parameters of the linear regression (slopes

on Ox, intercepts on Oy and correlation coefficients on Oz axes) is thus possible. The generated volumes indicate at which extent the comparison between different samples are distanced from the volume resulting through comparison of data sets belonging to an individual sample (for a more illustrative appearance, for individual tea samples analyzed 5 times repeatedly, one can chose the volume determined by the maximum variations of the linear regression parameters, on the three axes).

Such an evaluation manner drastically reduces the number of variables, from few thousands to only three (slope, intercept and correlation coefficient). Consequently, the experimental data sets may be generated with an unlimited number of items, which allows a highly accurate reproduction of the compared profiles.

For an easier interpretation of the comparisons being achieved, Figure 8 a-c illustrates the projections of the volumes being generated in the three planes (xOy, yOz, xOz) for samples analyzed through (+)MS.

One can easily observe that the approach allows discrimination among the five different tea types (each being analyzed 5 times consecutively). Surfaces resulting from comparison between T4-T5, and T2-T3, respectively, are placed in the immediate proximity of the surfaces determined by the comparison among the 5 successively repetitions for each of the 5 tea types , which is in good agreement with the HCA conclusions (grouping T4 and T5, T2 and T3, respectively).

Conclusions:

The method of the reduction of the experimental variables through consideration of the parameters of the linear regressions (slope, intercept and correlation coefficient) resulted from mutual comparison of the data sets produces information similar to chemometric PCA and HCA algorithms. The instrumental variability is not affecting the data interpretation methods.

For O4A1:

The possibility of large volume injection from supramolecular liquids (SUPRAS) was evaluated. Supramolecular liquids are already mentionned in the literature (F.J. Ruiz et al., Anal. Chem., 2007, 79, 7473-7484; A.B. Gomez et al., Anal. Chem., 2012, 84, 342-349; C. Burton et al., Anal. Chem., 2013, 85, 11137-11145) as being obtained from the tertiary systems water / tetrahydrofurane / non-ionic surfactant. More precisely, one discuss about coacervation of reversed micelles of alkanols or fatty acids in binary water/tetrahydrofurane mixtures. Supramolecular liquids formed through aggregation of the reversed micelles may be successfully used as extraction media with important applicative potential, because of their ability to retain polar small analytes in the aqueous media inside the micelle, apolar small analytes in the walls of the micelle, but exhibiting a size exclusion effect towards compounds with a higher molecular mass (i.e. proteins, humic acids, polysaccharides). The interest of using supramolecular liquids as extracting media is high, if considering environmental or bioanalytical applications (complicated matrices).

The sensitivity of all analytical approaches is directly related to the quantity (or volume) of the sample being considered for analysis. It is why a study dedicated to the possibility of large volume injection of supramolecular liquids in RPLC is a subject of interest.

The effect of LVI of samples containing carbamazepine dissolved in water, tetrahydrofurane, octanol and the supramolecular solvent prepared in the system water/THF/octanol was studied. The chromatographic separation was achieved on an ODS column (5 cm L × 4.6 mm i.d. × 1.8 μm d.p.), using a mobile phase containing an aqueous formic acid solution 0.1% (v/v) and acetonitrile. The flow rate was 0.6 mL/min and the column was maintained at 25 °C. UV detection was applied at 254 nm. The injected volumes were 1, 5, 10, 20, 50 μL, keeping the injected of carbamazepine amount of 1 μg. Isocratic conditions were used, consisting in 75% organic modifier in the mobile phase. Some gradient elution conditions were also used (10% organic modifier for 1 min, next to 100% in 6 min, were kept for 5 min). Solutions of carbamazepine in water, THF, octanol and SUPRAS were injected. The results are presented in Figure 9-a-e. The SUPRAS was obtained through mixing 1.5 mL of the aqueous carbamazepine solution to 1.5 mL THF and 0.35 mL octanol.

One can observe that THF, if used as solvent, allowed the injection of a miximum volume of 1 µL. Solutions in octanol may be injected up to 20 µL, in isocratic elution. In similar conditions, the maximum SUPRAS injected volume may be 10 µL. Same limits may be observed in gradient elution conditions.

Conclusions:

Relative large volumes of SUPRAS may be loaded to RPLC columns (we should consider that for the column being used recommended injection volumes are up to 2 µL). These preliminary results indicate that the use of SUPRAS as extraction media to be loaded directly to RPLC columns is possible, opening an interesting potential in applications dealing with very important matrix effects from the environmental and bioanalysis fields.

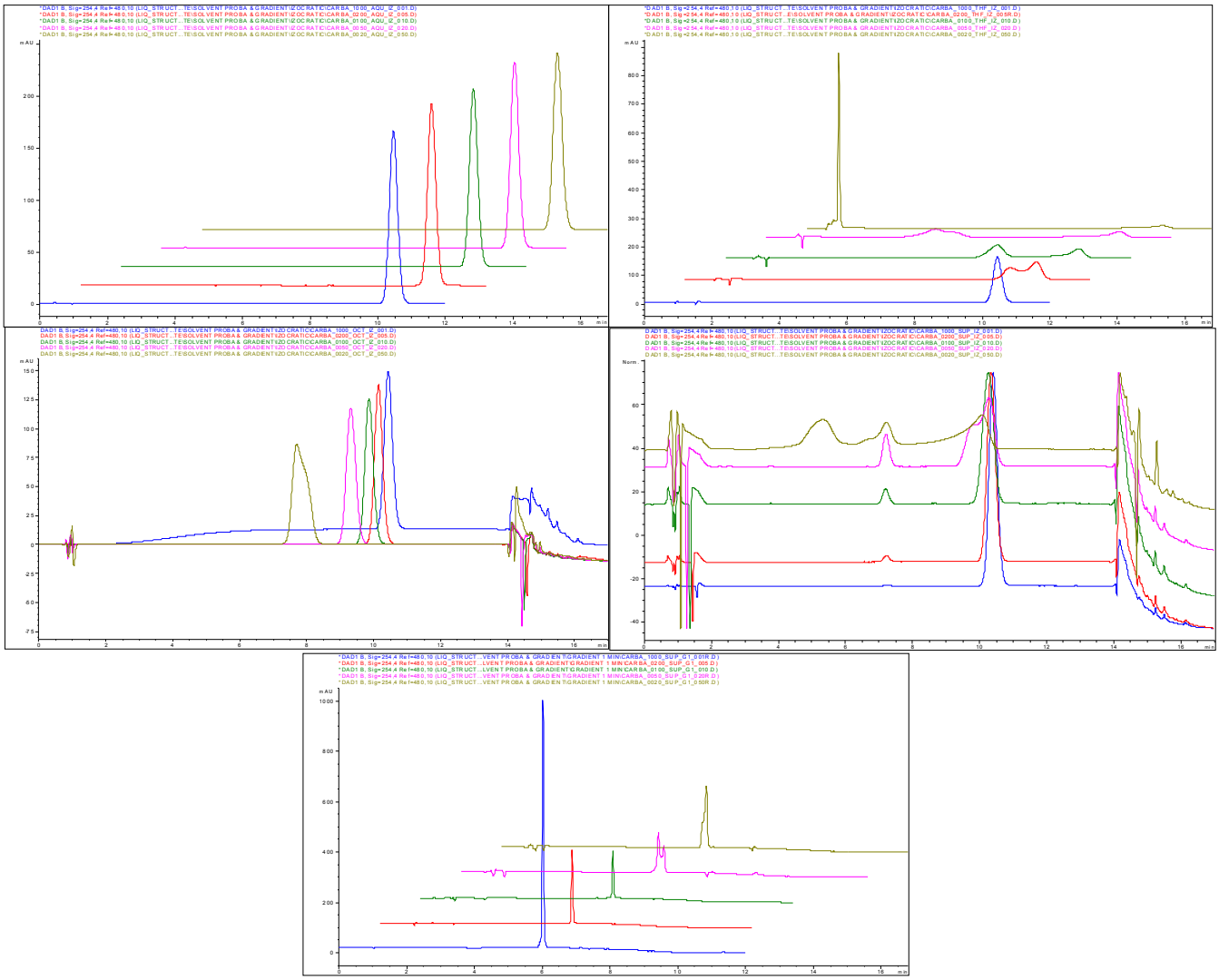


Figure 9. Increasing volumes of 1 µg carbamazepine in (a0 water; (b) THF; (c) octanol; (d) SUPRAS in isocratic and (e) gradient elution conditions.

General conclusions

During the period December 16th, 2014 –December 15th, 2015, the project team realized 2 ISI publications, as it follows:

1. P. Lazăr, Ş. Udrescu, F. Tache, F. Albu, N. Grinberg, A. Medvedovici, Revisiting large volume injection in non-miscible diluents: an on-line reversed phase supported liquid extraction / liquid chromatography scenario, Analytical Methods (2015) 7(1) 342-352. I.F.=1.821

2. F. Micăle, F. Albu, E.E. Iorgulescu, A. Medvedovici, F. Tache, Ethyl lactate as a greener alternative to acetonitrile in RPLC: A realistic appraisal, Journal of Chromatographic Science (2015) 53(10) 1701-1707. I.F.=1.363

The results presented in the above mentionned publications correspond to the following objectives (O) and activities (A) of the project:

Publication 1. O4A1;
Publication 2. O1A2; O2A1;

The dissemination of the results being obtained throughout the project plan completion was also achieved:

Symposium 1. International Workshop "Food Chemistry & Engineering", Ovidius University of Constanța, Faculty of Applied Sciences and Engineering - Romania, Mai 15-16, 2015; plenary lecture: Green chromatographic assay of PAHs in dietary supplements and foodstuff (A. Medvedovici, F. Micăle, F. Tache), Book of Abstracts: KN1 - pg. 7.

Symposium 2. 1st International Conference "From science to guidance and practice„ 19-21 Octombrie 2015, Bucharest - Romania; plenary lecture: Chromatographic approaches for evaluation of the hydrophobic characteristics of chemical compounds with emphasis on newly developed separation mechanisms (A. Medvedovici, V. Voicu), Book of Abstracts: - pg. 1, 56. ISBN: 978-973-708-854-3.

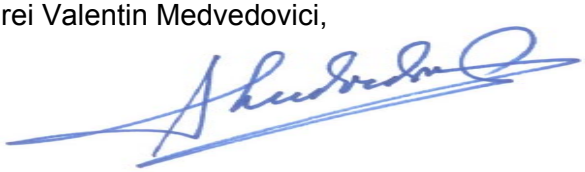
Publications and conferences above mentioned are contributing to objectives O5A1, O5A2 of the project, referring to dissemination of the research results. Above mentioned publications include in the section *Acknowledgements* the indicative of the present grant. Power Point presentations used during oral presentations are also containing in the last slide the indicative of the present grant.

Publications (first page and the page with the Acknowledgements section) were posted on the project site http://www.chimie.unibuc.ro/cercetare/analitica /lvihplc/index.htm. Power Point presentations are also posted (in their entirety) on the same site.

Management and permanent up-dating of the project site responds equally to Objective 5.

The experimental data, the published manuscripts and plenary lectures contributing to the dissemination of results comply to the objectives and activities redistributed according to project rescheduling plan (Annex IV of the financing contract nr. 310/2011). The Romanian version of the present document was up-loaded on the UEFISCDI site.

Project Director,
Professor Dr. Andrei Valentin Medvedovici,



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