Activity report

Concerning the Project PN-II-ID-PCE-2011-3-0152

entitled "Large Volume Injection (LVI) of Mobile Phase Immiscible Diluents in Liquid Chromatrography (LC)"

Contract No. 310/05.10.2011,

having as Director Prof. Dr. Andrei Medvedovici, from the University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry

Period covered by the report: 05.10.2011 - 04.11.2016

Summary of the Objectives (O) and subsequent Activities (A) covered by the project:

Objective 1: Phenomenological insights about the LVI of immiscible diluent in LC.

A1. Checking for diluents;

A2. Checking for diluent mixtures;

A3. Checking on influence of temperature;

A4. Checking on the flow rate influence;

A5. Checking for different analyte classes;

A6. Checking for different stationary phases;

A7. Checking for different stationary phases morphologies;

A8. Checking for polymeric stationary phases;

A9. Checking for organic modifiers in mobile phases accommodating LVI of immiscible diluents;

A10. Checking for different separation mechanisms.

Objective 2: Proposal of an adsorption model of the diluent.

A1. Study of the elution shape of the diluent front;

Objective 3: Characterization of the hydrophobic character of solutes based on their ability to perform LVI.

A1. Series of immiscible diluents for progressive log P scales of target analytes;

A2. Identification of general conditions needed for LVI in immiscible diluents, based on comparison of the hydrophobic character of diluents and analytes;

A3. Correlation between the hydrophobic characteristics of the analytes and the LVI in immiscible diluents process;

Objective 4: Identification of green solvents to be used for sample preparation further performing a role in the LVI of immiscible diluents in LC.

Objective 5: On-line automation issues in LVI of immiscible diluents for LC.

A1. LLE/LVI/LC issue;

A2. SPE/LVI/LC issue;

Objective 6: Identification of applications in various fields.

A1. Bioanalytical applications;

A2. Quality control in pharmaceutics;

A3. Environmental applications;

A4. Food chemistry applications.

Objective 7: Dissemination of the results.

A1. ISI publications;

A2. Participation to conferences and Symposia.

Objective 8: Web page of the project: creation and up-grading.

The project also aimed to allow acquisition of analytical equipments, as it follows:

Equipment to be acquired within the project:

1. Auto-sampler with LVI kit (5-900 μL).
2. Refractive Index Detector.
3. Ultra high pressure binary HPLC pump (UPLC pump).
4. UV-Vis detector.
5. Column thermostat.
6. Vortex
7. Computers (x 3) and printer (x1)
8. Refrigerator (for storage of temperature sensitive samples).

Results:

The study of the intrinsic processes leading to the possibility of using LVI of immiscible diluents in reversed phase liquid chromatography (RPLC) was presented in the Scientific Reports (2011, 2012, 2013, 2014, 2015 and 2016), Romanian versions loaded to the web page of the project (http://www.chimie.unibuc.ro/cercetare/analitica/lvihplc/index.htm). These scientific reports covered **O1/A1-A10**. By cumulating the knowledge acquired during the performed experiments, we proposed a new mechanism explaining LVI of immiscible diluents in RPLC. A mathematical model (**O2/A1**), some thermodynamic approaches and a hydrophobicity indices scale derived from the process (**O3/A1-3**) were presented in Publication 3. The proposed mechanism illustrates LVI of immiscible diluents in RPLC as an on-line coupled process between Reversed Phase Supported Liquid Extraction (RP-SLE) and RPLC (covering aspects under **O5/A1-A2**).

As a recognition of the major role of LVI in immiscible solvents and its application in bioanalysis (**O6/A1**), a review on the topic has been required by the journal Bioanalysis (see Publication 6). The same journal hosted our publication with respect to the assay of bis-quaternary

pyridinium oximes in biological matrices, representing an application of the project topic for bioanalysis (**O6/A1**) - see Publication 7. A comparative overview of the topic's application in the antidoping field (**O6/A1**) has been published in Revue Roumaine de Chimie, the issue dedicated to the 100th anniversary from the birth date of Prof. Dr. Candin Liteanu (see Publication 4). Bioanalytical topics were also detailed in Publications 8 and 11.

Aspects covering **O6/A2**, referring to the application of the research topic in the domain of the quality control in the pharmaceutical industry are addressed in the Publications 10 and 12.

Applications relating to the food chemistry domain (**O6/A4**) are contained in Publications 1 and 5.

Applications of the studied topic in the field of green chromatographic approaches (**O4**) have been materialized in Publications 2, 8 and 9.

To conclude, the research carried out under the frame of the project was materialized in 12 ISI publications, summarizing a cumulated impact factor (C.I.F) of 27.785. The published papers were distributed as it follows: 2012 / 1; 2013 / 6; 2014 / 2, 2015 / 2, 2016 / 1. Two of these papers were puplished in journals classified in the red zone according to AIS (Talanta), and six were puplished in journals classified in the yellow zone (2 x Bioanalysis, 2 x Journal of Pharmaceutical and Biomedical Analysis, 1 x Journal of Chromatography B, 1 x Analytical Methods). These results are fulfilling criteria existing on the electronic platform of UEFISCDI, Planning of the Project Results, in which one ISI publication was planned for 2012, 2 ISI publications were planned for 2013, 1 for 2014, 1 for 2015 and 1 for 2016. These publications accounts for **O7/A1**.

The resulting publications also illustrated the collaboration of the project team with other research teams from Romania (the team of Professor Dr. Victor Voicu, from the University of Medicine and Pharmacy "Carol Davila", Bucharest, the team of Prof. Dr. Victor David from the University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry; the team of Prof. Dr. Costel Sârbu from the University Babeș-Bolyai, Faculty of Chemistry and Chemical Engineering, Cluj-Napoca) and from abroad (the team of Nelu Grinberg from Boehringer Ingelheim, U.S.A. and the team of Professors Dr. Kuca and Musilek, from the University of Hradec Kralove, Czech Republic).

According to **O7/A2**, the findings obtained under the framework of the project should be also disseminated through participation to conferences and symposia. The list of the plenary lectures contained herein (**Plenary lectures list**) illustrate the participation of the project team to 14 scientific events including 12 international conferences or symposia and 2 national ones. Distribution over the project period is: 2011 / 1; 2012 / 3; 2013 / 2; 2014 / 3, 2015 / 2, 2016 / 3.

With respect to the analytical equipment acquired over the project development, the list is as following:

Vortex mixer and accessories:	acquisition date 27.07.2016 (invoice no. 341)
Evaporator/heater and accessories:	acquisition date 27.07.2016 (invoice no. 341)
DAD up-grading kit:	acquisition date 09.09.2015 (invoice no. 6193)

Auto-sampler:	acquisition date 13.09.2013 (invoice no. 0003322).
LVI kit:	acquisition date 26.09.2012 (invoice no. 0003086).
Refractive Index Detector (RID):	acquisition date 26.09.2012 (invoice no. 0003086).
UPLC pump:	acquisition date 26.09.2012 (invoice no. 0003086).
UV-Vis detector:	acquisition date 09.07.2013 (invoice no. 0003293).
Column thermostat:	acquisition date 28.07.2014 (invoice no. 0003491).
Computer (3 laptops):	acquisition date 08.11.2011 (invoice no. 91404942).
Refrigerator:	acquisition date 19.07.2012 (invoice no. 1201178).

As a final conclusion, all activities carried out under the framework of the PN-II-ID-PCE-2011-3-0152 project fulfilled (and even overcame) the requirements of the project master plan.

Publication List

Publication 1. E. Iorgulescu, V.A. Voicu, C. Sârbu, F. Tache, F. Albu, A. Medvedovici, Experimental variability and data pre-processing as factors affecting the discrimination power of some chemometric approaches(PCA, CA and a new algorithm based on linear regression) applied to (±) ESI/MS and RPLC/UV data: Application on green tea extracts, ***Talanta*** 155 (2016) 133-144 (I.F. = 3,545).

Publication 2. F. Micăle, F. Albu, E.E. Iorgulescu, A. Medvedovici, F. Tache, Ethyl lactate as a greener alternative to acetonitrile in RPLC: A realistic uppraisal, ***Journal of Chromatographic Science*** 53(10) (2015) 1701-1707 (I.F. = 1,320).

Publication 3. P. Lazăr, Ș. 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*** 7 (2015) 342-352 (I.F. = 1,821).

Publication 4. E.E. Iorgulescu, F. Albu, F. Tache, A. Medvedovici, On the HPLC/MS-MS of ephedrines in urine: an experimental appraisal, ***Revue Roumaine de Chimie***, 59(11-12) (2014) 1047-1057. (I.F.=0.311).

Publication 5. A. Medvedovici, F. Albu, R.-D. Nașcu-Briciu, C. Sârbu, Fuzzy clustering evaluation of the discrimnination power of UV-Vis and (+/-) ESI-MS detection system in individual or coupled RPLC for characterization of Ginkgo Biloba standardized extracts, ***Talanta*** 119 (2014) 524-532 (I.F.=3.545).

Publication 6. V. David, M. Cheregi, A. Medvedovici, Alternative sample diluents in bioanalytical LC-MS, ***Bioanalysis*** 5(24) (2013) 3051-3061 (I.F. = 2.813).

Publication 7. V. Voicu, F. Albu, F. Tache, K. Musilek, K. Kuca, A. Medvedovici, LC-MS/MS approaches for the assay of bis-quaternary pyridinium oximes used as AChE reactivators in biological matrices, ***Bioanalysis*** 5(7) (2013) 793-809 (I.F. = 2.813).

Publication 8. M. Cheregi, F. Albu, Ș. Udrescu, N. Răducanu, A. Medvedovici, Greener bioanalytical approach for LC/MS-MS assay of enalapril and enalaprilat in human plasma with total replacement of acetonitrile throughout all analytical stages, ***Journal of Chromatography B*** 927 (2013) 124-132 (I.F.=2.729).

Publication 9. F. Tache, Ș. Udrescu, F. Albu, F. Micăle, A. Medvedovici, Greening pharmaceutical applications of liquid chromatography through using propylene carbonate-ethanol mixtures instead of acetonitrile as organic modifier in the mobile phases, ***Journal of Pharmaceutical and Biomedical Analysis*** 75 (2013) 230-238 (I.F.=2.979).

Publication 10. N. Grinberg, F. Albu, K. Fandrick, E.E. Iorgulescu, A. Medvedovici, Assay at low ppm level of dimethyl sulfate in starting materials for APIs synthesis using derivatization in ionic liquid media and LC-MS, ***Journal of Pharmaceutical and Biomedical Analysis*** 75 (2013) 1-6 (I.F.=2.979).

Publication 11. A. Medvedovici, Ș. Udrescu, V. David, Use of a green (bio)solvent - limonene - as extractant in RPLC - tandem MS assay of statins and related metabolites in human plasma, ***Biomedical Chromatography*** 27(1) (2013) 48-57 (I.F.=1.723).

Publication 12. T. Galaon, M. Rădulescu, V. David, A. Medvedovici, Use of an immiscible diluent in ionic liquid/ion pair LC for the assay of an injectable analgesic, ***Central European Journal of Chemistry*** 10(4) (2012) 1360-1368 (I.F.=1.207).

Plenary lectures (PL) list

PL1. ***Large volume injection of sample diluents non-miscible with the mobile phase in RPLC: principles and applications***; *A. Medvedovici*;, 17th International Symposium in Separation Science, September 5-9th, Cluj-Napoca, Romania, 2011.

PL2. ***Green solution for replacement of acetonitrile as mobile phase component in RPLC separations***, *A. Medvedovici*, F. Tache, Ș. Udrescu; International Conference CHIMIA 2012 – New trends in Applied Chemistry, May 24-26th, Constanța, Romania, 2012.

PL3. ***New approaches for the bioassay of oximes by means of LC-ESI/MS/MS***; *A. Medvedovici*, F.Albu, V. Voicu; 13th International Congress of Romanian Society of Clinical Pharmacology, Therapeutics and Toxicology, June 11-14th, Poiana Brașov, Romania, 2012.

PL4. ***Green approach in bioassay through replacement of acetonitrile from sample preparation and separation stages***; *A. Medvedovici*, F. Albu; 1st International Conference on Analytical Chemistry, RO-ICAC 2012; September 18-21st, Târgoviște, Romania, 2012.

PL5. ***Comprehesivity and hyphenation in analysis of fats***; *A. Medvedovici*; International Workshop "Challenges in Food Chemistry", May 31st - June 1st, Constanța, Romania, 2013.

PL6. ***LVI of non-miscible diluents in RPLC or simply on-line RP-SLE/RPLC? – Principles and Applications***; *A. Medvedovici*, P. Lazăr, Ș. Udrescu; Inaugural Workshop of the Romanian Chapter of the American Chemical Society, December 13th, Bucharest, Romania, 2013.

PL7. ***On-line Reversed Phase Supported Liquid Extraction (RP-SLE) as a basic scenario for explaining large volume injection (LVI) of immiscible diluents in RPLC***; *A. Medvedovici*, P. Lazăr; CHIMIA 2014 – New trends in Applied Chemistry, May 23-24th, Constanta, Romania, 2014.

PL8. ***Actual aspects in Separation Sciences***; *A. Medvedovici*, Micro-symposium dedicated to commemoration of one century from the birth of Prof. Dr. Candin Liteanu, September 11th, Cluj-Napoca, Romania 2014.

PL9. ***Hydrophobicity descriptors resulting from bimodal LC behavior (RP and HILIC)***; *A. Medvedovici*; 2nd International Conference on Analytical Chemistry, RO-ICAC 2014; September 17-21st, Târgoviște, Romania, 2014.

PL10. ***Green chromatographic assay of PAHs in dietary supplements and foodstuff***; A. Medvedovici, F. Micale, F. Tache; International Workshop "Food Chemistry & Engineering"; May 15th, Constanta, Romania, 2015.

PL11. **Chromatographic approaches for evaluation of the hydrophobic characteristics of chemicals with emphasis on newly developed separation mechanisms**; A. Medvedovici, V. Voicu; International Conference "From Science to Guidance and Practice" October, 19th-21st, Bucharest, Romania, 2015.

PL12. ***New chemometric approach based on linear regression for holistic comparison of analytical data***; A. Medvedovici; International Conference Chimia 2016, New Trends in Applied Chemistry, May 26th-28th, Constanta, Romania, 2016.

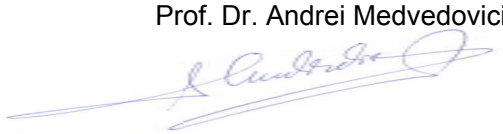
PL13. ***Chemometric approaches for characterization of complex mixtures of natural origins used as active ingredients in dietary supplements***; A. Medvedovici, V. Voicu; International Conference "From Science to Guidance and Practice" June, 6th-7th, Bucharest, Romania, 2016.

PL14. ***Application of liophilic additives in RPLC mobile phases***, A. Medvedovici, Advances and Application in Dissolution Sciences, Disso-Europe 2016, October 20th-21st, Bucharest, Romania, 2016.

October 10th, 2016

Director of the project,

Prof. Dr. Andrei Medvedovici



Faculty of Chemistry
University of Bucharest
Panduri Ave. no. 90-92,
Bucharest-050663, Romania
E-mail: avmedved@yahoo.com
Fax: 021 4102279
Tel.: 072 3330423