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SOLVENT MIXTURES TOOL FOR SEPARATION OF BIOLOGICAL ACTIVE COMPOUNDS

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Introduction. Chromatographic mobile phase mixtures offer a great opportunity for better analytical separation in both qualitative TLC and quantitative HPLC methods. The chromatographic mobile phase preparation involves a numeric taxonomy procedure for mixture constituents selecting, based on solvent strengths, and an optimization of its composition based on a series of factorial analysis designed experiments.

Materials. At least two variables are involved when we try to optimize a solvent mixture. And here, let us to assume that we want to use a mixture of three solvents, which we believe that is capable to provide satisfactory results in terms of reparability of our compounds mixture. Thus, our variables are solvents mixture composition and compounds mixture composition.

In terms of qualitative measurements, we are interested which one compounds are in our mixture. In terms of quantitative measurements, we want to find a proper solvent mixture (i.e. solvent mixture composition) in order to obtain the best reparability of these compounds in order to be determined quantitatively. A difficulty occurs when our compounds behavior is similar, such when are from same chemical class, the chromatographic separation being more difficult to do then. Our subject of investigation was mixtures of compounds, each one from following classes: steroids, androstane isomers, hydrophilic vitamins, N-alkyl phenothiazine sulfones, and benzodiazepines. **Method.** Elaborating of mathematical models trough embedding of mathematical equations stays at the fundament of surface properties of liquids and repartition and distribution equations between phases, has relevant implications for characterization of biologically active compounds, but until now, still few researches are regarded to this subject, dealing with different situations from one mixture of compounds to another and from one mixture of solvents to another. A factorial analysis is suggested as alternative in these cases. Are assumed that in a mixture of three solvents the quantitative measure of choused chromatographic parameter depends on mobile phase composition through a dependency equation of one of the types: $M6(x_1,x_2,x_3) = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3$ **(eq1)** and $M7(x_1,x_2,x_3) = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + a_7x_1x_2x_3$ **(eq2)**, where x_1, x_2, x_3 are the molar fractions of the solvents ($x_1 + x_2 + x_3 = 1$), M6 and M7 are estimators and then predictors of choused chromatographic parameter and a_1, a_2, a_3, a_4, a_5, a_6 and a_7 are the coefficients which are first obtained using the best fit of chromatographic parameter and second used to predict the values of this parameter for any composition of mobile phase. Parameters which was modeled using (eq1) and (eq2) was:

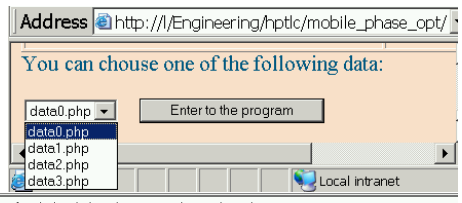
- $R_f = R_f(i,e) = l(i)/l(e)$, where *i* is one compound to be separated by use of the *e* eluent, *l(i)* is the migration distance of the compound in the eluent, *l(e)* is the migration distance of the eluent, and R_f the array of retention factors of to be separated components in the eluent *e*.
- $R_s(i,j,e) = 2(l(i)-l(j))/(w(i)+w(j))$, where *i* and *j* are two to be separated compounds, *w(i)* and *w(j)* are zones widths at baseline, and R_s is the matrix of resolutions between *i* and *j* compounds.
- $R_{so}(i,e) = 2(l_o(i)-l_o(i+1))/(w_o(i)+w_o(i+1))$, where $l_o(i)$ is the *i*-th migration coordinate in the *ordered list* of migration lengths, w_o is the corresponding witdh, and R_{so} is the matrix of resolutions for consecutive migrated compounds.
- $IP(e) = \sqrt{\sum_j(\Delta R_{f,t}-\Delta R_f(j,e))^2}/\sqrt{n(n+1)}$, where *n* is the number of compounds to be separated, $\Delta R_{f,t}$ is the ideal retention factor (1/n), $\Delta R_f(j,e)$ the *j*-th difference of retention factors between two consecutive migrated compounds, and *IP* is a separation mean descriptor recorded for the eluent *e*.
- $R_{sa}(e) = \sum_j R_{so}(j,e)/(n-1)$, where R_{sa} is the averaged resolution of separation by use of the eluent *e*.
- $RRP(e) = \prod_j R_{so}(j,e)/R_{sa}(e)$, where *RRP* being the relative resolutions on the eluent *e* product type descriptor.
- $Inf(e,m) = \sum_k (n_k/n)\log_2(n_k/n)$, where n_k being the number of compounds which migrates in the *k*-th equidistant interval from the total number of *m* in which was divided the whole migration length and *Inf* are a quality factor computed according to *Logit* method and are null for a ideal separation.
- $F_{ob}(e,m) = \sum_j a_j F_j(IP(e),Inf(e,m),R_{sa}(e),RRP(e))$, where $1 \le j \le 4$ (for five compounds to be separated), F_j are functions which express every one a composed expression of all four chromatographic parameters (*IP(e)*, *Inf(e,m)*, $R_{sa}(e)$, *RRP(e)*), a_j are coefficients choused through a weighted relationship mathematically defined, and F_{ob} is a objective function which characterizes the separation in the eluent e according with chousing of the a_j coefficients, F_j functions, and number of equidistance intervals, *m*.
- Through applying of one of Eq2-9 for a array of *p* experiments it results a matrix, M_{ob} with one (EqX) or more rows (EqY) and always *p* columns, each one for every experiment, of which elements represents the values of the modeled chromatographic parameter (by use of the Eq1).

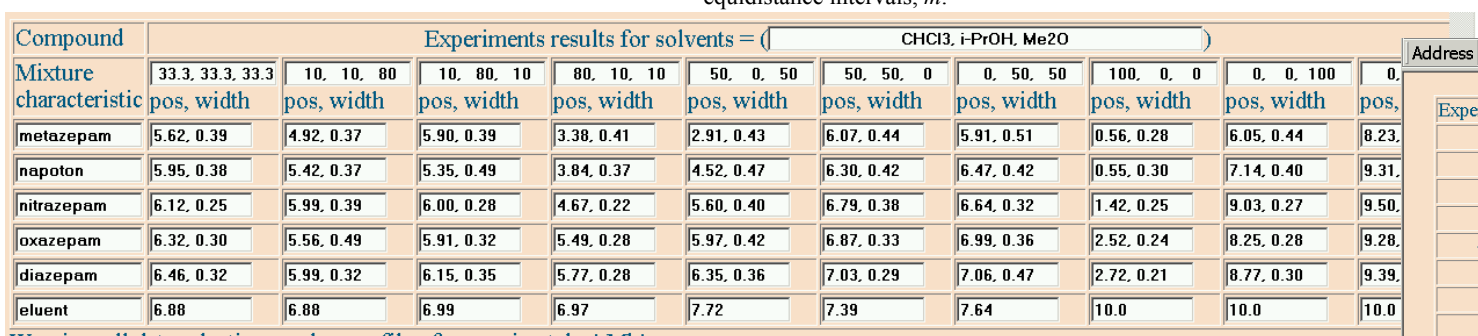
The imposed prerequisite of the Eq1 in order to the optimization algorithm to provide a unique determined solution is at least that $p \ge 7$.

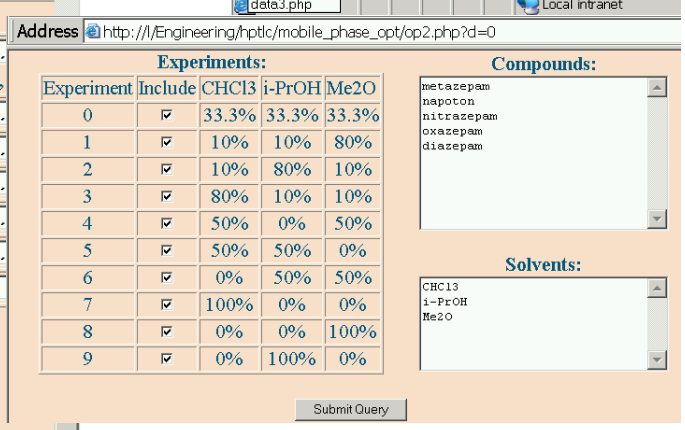
Remark: Our software is open access and free to use.

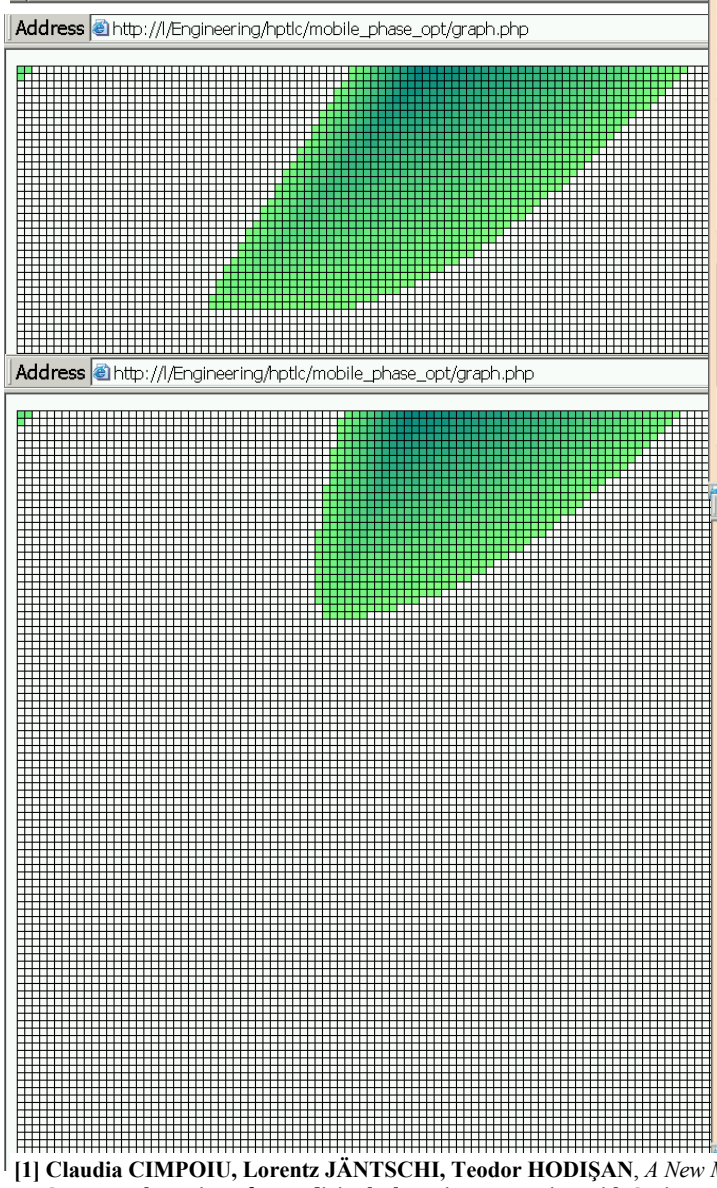
Results: Software implementation. A software embedding the mathematical model were build and were published online (following are screen captures from program execution:

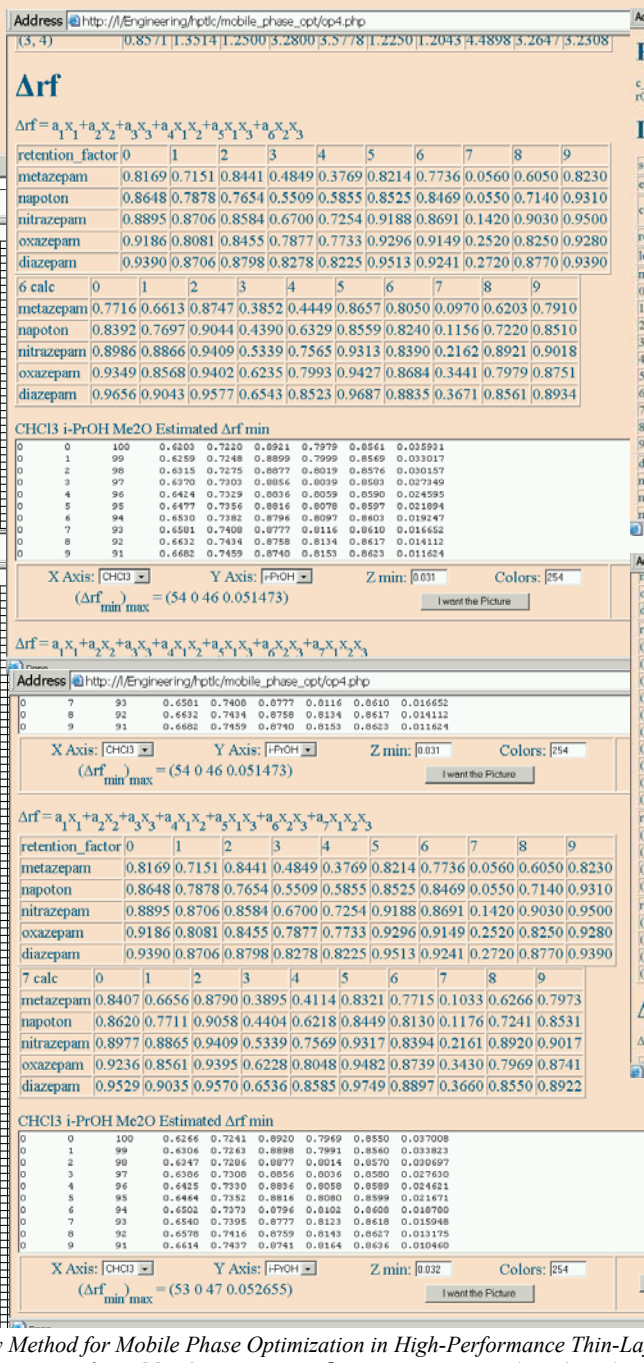
http://l.academicdirect.org/Engineering/hptlc/mobile_phase_opt/

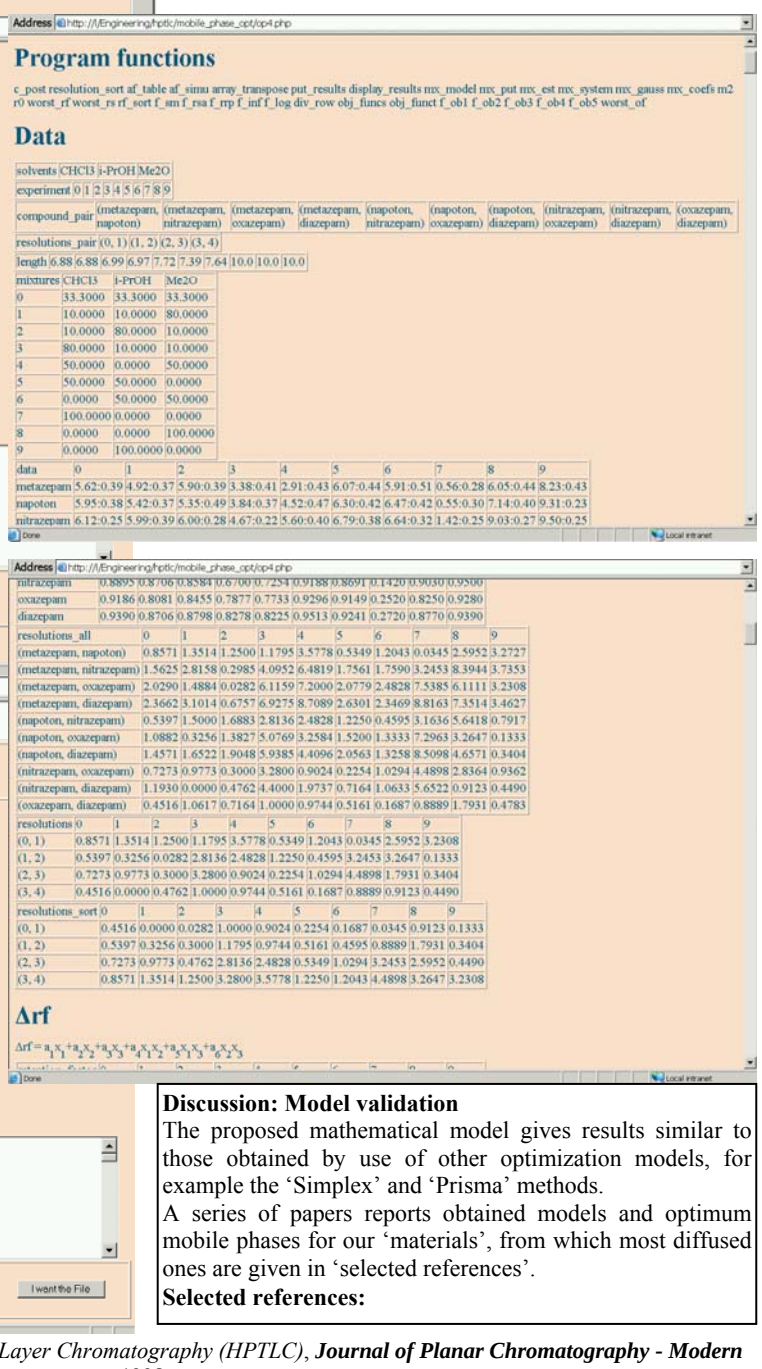


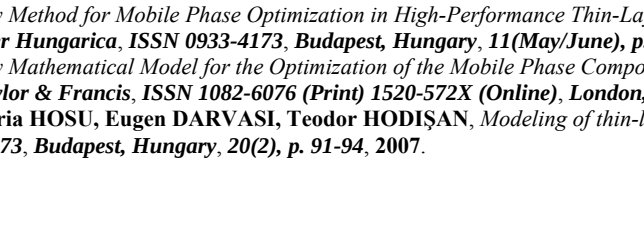












Discussion: Model validation

The proposed mathematical model gives results similar to those obtained by use of other optimization models, for example the 'Simplex' and 'Prisma' methods.

A series of papers reports obtained models and optimum mobile phases for our 'materials', from which most diffused ones are given in 'selected references'.

Selected references:

[1] Claudia CIMPOIU, Lorentz JÄNTSCHI, Teodor HODIŞAN, *A New Method for Mobile Phase Optimization in High-Performance Thin-Layer Chromatography (HPTLC)*, ***Journal of Planar Chromatography - Modern TLC***, ***Research Institute for Medicinal Plants in cooperation with Springer Hungarica***, ***ISSN 0933-4173***, ***Budapest, Hungary***, ***11(May/June), p. 191-194***, **1998**.

[2] Claudia CIMPOIU, Lorentz JÄNTSCHI, Teodor HODIŞAN, *A New Mathematical Model for the Optimization of the Mobile Phase Composition in HPTLC and the Comparision with Other Models*, ***Journal of Liquid Chromatography and Related Technologies***, ***Marcel Dekker Inc. then Taylor & Francis***, ***ISSN 1082-6076 (Print) 1520-572X (Online)***, ***London, England***, ***22(10), p. 1429-1441***, **1999**.

[3] Lorentz JÄNTSCHI, Sorin HODIŞAN, Claudia CIMPOIU, Anamaria HOSU, Eugen DARVASI, Teodor HODIŞAN, *Modeling of thin-layer chromatographic separation of androstane isomers*, ***JPC - Journal of Planar Chromatography - Modern TLC***, ***Akadémiai Kiadó***, ***ISSN 0933-4173***, ***Budapest, Hungary***, ***20(2), p. 91-94***, **2007**.