



# 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 through 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) are:

- $R_f = R_f(i,e) = l(i)/l(e)$ , 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.
- $R_{so}(i,e) = 2(l_0(i)-l_0(i+1))/(w_0(i)+w_0(i+1))$ , where  $l_0(i)$  is the  $i$ -th migration coordinate in the ordered list of migration lengths,  $w_0$  is the corresponding width, and  $R_{so}$  is the matrix of resolutions for consecutive migrated compounds.
- $IP(e) = \sqrt{\sum(\Delta R_{f,i} - \Delta R_{f,j}(e))^2/n(n+1)}$ , where  $n$  is the number of compounds to be separated,  $\Delta R_{f,i}$  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  $1 \leq j \leq 4$  (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  $i$  (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  $p \geq 7$ .

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

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

The screenshot shows the main interface of the software. At the top, there is a navigation bar with the address [http://Engineering/hptlc/mobile\\_phase\\_opt/](http://Engineering/hptlc/mobile_phase_opt/). Below this, a message says "You can choose one of the following data:" followed by a dropdown menu with options: data0.php, data1.php, data2.php, data3.php. There is an "Enter to the program" button. On the right, there are sections for "Compounds:" and "Solvents:". The "Compounds:" section lists metazepam, napoton, nitrazepam, oxazepam, diazepam. The "Solvents:" section lists CHCl3, i-PrOH, Me2O. Below these, there is a table for "Experiments:" with columns for Experiment, Include, CHCl3, i-PrOH, Me2O. The table shows 9 experiments with checkboxes for inclusion and percentage values for each solvent. A "Submit Query" button is at the bottom.

Compound	Experiments results for solvents = ( CHCl3, i-PrOH, Me2O )											
	33.3, 33.3, 33.3	10, 10, 80	10, 80, 10	80, 10, 10	50, 0, 50	50, 50, 0	0, 50, 50	100, 0, 0	0, 0, 100	0, 0, 100	0, 0, 100	0, 0, 100
Mixture characteristic	pos, width	pos, width	pos, width	pos, width	pos, width	pos, width	pos, width	pos, width	pos, width	pos, width	pos, width	pos, width
metazepam	5.62, 0.39	4.92, 0.37	5.90, 0.39	3.38, 0.41	2.91, 0.43	6.07, 0.44	5.91, 0.51	0.56, 0.28	6.05, 0.44	6.05, 0.44	6.05, 0.44	8.23
napoton	5.95, 0.38	5.42, 0.37	5.35, 0.49	3.84, 0.37	4.52, 0.47	6.30, 0.42	6.47, 0.42	0.55, 0.30	7.14, 0.40	7.14, 0.40	7.14, 0.40	9.31
nitrazepam	6.12, 0.25	5.99, 0.39	6.00, 0.28	4.67, 0.22	5.60, 0.40	6.79, 0.38	6.64, 0.32	1.42, 0.25	9.03, 0.27	9.03, 0.27	9.03, 0.27	9.50
oxazepam	6.32, 0.30	5.56, 0.49	5.91, 0.32	5.49, 0.28	5.97, 0.42	6.87, 0.33	6.99, 0.36	2.52, 0.24	8.25, 0.28	8.25, 0.28	8.25, 0.28	9.28
diazepam	6.46, 0.32	5.99, 0.32	6.15, 0.35	5.77, 0.28	6.35, 0.36	7.03, 0.29	7.06, 0.47	2.72, 0.21	8.77, 0.30	8.77, 0.30	8.77, 0.30	9.39
eluent	6.88	6.88	6.99	6.97	7.72	7.39	7.64	10.0	10.0	10.0	10.0	10.0

Warning: all data selection produces a file of approximately 4 Mb!

Model Equation:

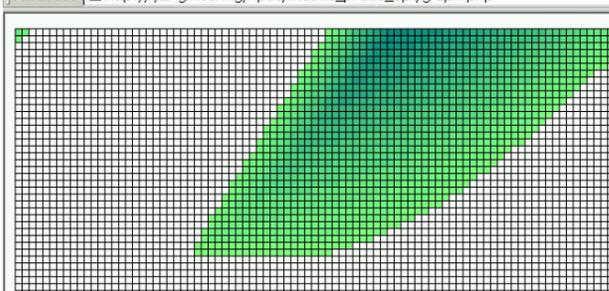
- $Y = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3$
- $Y = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_1x_2 + a_5x_1x_3 + a_6x_2x_3 + a_7x_1x_2x_3$

Dependent Variable:  FO  RS  RF

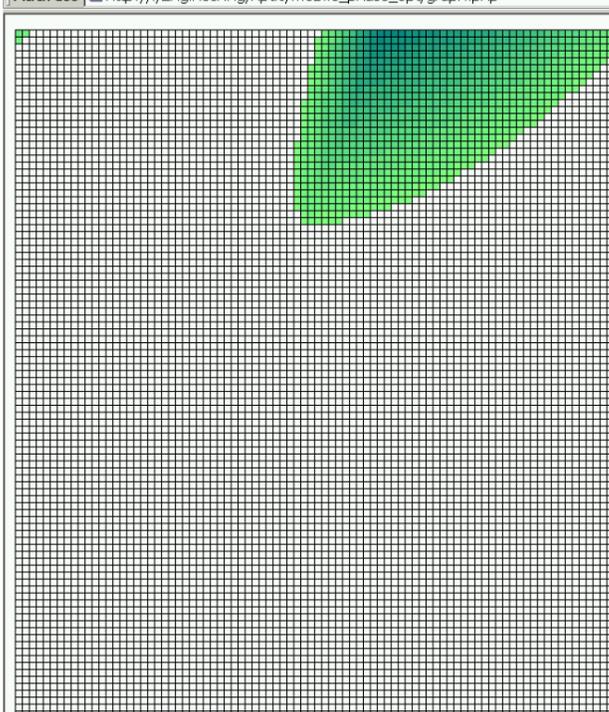
Graph:  Red (best) - Green (worst)  Green gradient only

Submit Query

Address [http://Engineering/hptlc/mobile\\_phase\\_opt/graph.php](http://Engineering/hptlc/mobile_phase_opt/graph.php)



Address [http://Engineering/hptlc/mobile\\_phase\\_opt/graph.php](http://Engineering/hptlc/mobile_phase_opt/graph.php)



Address [http://Engineering/hptlc/mobile\\_phase\\_opt/sp4.php](http://Engineering/hptlc/mobile_phase_opt/sp4.php)

Program functions

Data

solvents CHCl3 i-PrOH Me2O

experiment 0 1 2 3 4 5 6 7 8 9

compound\_pair (metazepam, metazepam), (metazepam, napoton), (metazepam, nitrazepam), (metazepam, oxazepam), (metazepam, diazepam), (napoton, napoton), (napoton, nitrazepam), (napoton, oxazepam), (nitrazepam, nitrazepam), (nitrazepam, oxazepam), (diazepam, diazepam), (diazepam, oxazepam)

resolutions\_pair (0, 1), (1, 2), (2, 3), (3, 4)

length 6.88 6.88 6.99 6.97 7.72 7.39 7.64 10.0 10.0 10.0

mixtures CHCl3 i-PrOH Me2O

0	1	2	3	4	5	6	7	8	9	
metazepam	0.7716	0.6613	0.8747	0.3852	0.4449	0.8657	0.8050	0.0970	0.6203	0.7910
napoton	0.8392	0.7697	0.9044	0.4390	0.6329	0.8559	0.8240	0.1156	0.7220	0.8510
nitrazepam	0.8986	0.8866	0.9409	0.5339	0.7565	0.9313	0.8390	0.2162	0.8921	0.9018
oxazepam	0.9349	0.8568	0.9402	0.6235	0.7993	0.9427	0.8684	0.3441	0.7979	0.8751
diazepam	0.9656	0.9043	0.9577	0.6543	0.8523	0.9687	0.8835	0.3671	0.8561	0.8934

CHCl3 i-PrOH Me2O Estimated Arf min

0	1	2	3	4	5	6	7	8	9	
metazepam	0.8169	0.7151	0.8441	0.4849	0.3769	0.8214	0.7736	0.0560	0.6050	0.8230
napoton	0.8648	0.7878	0.7654	0.5509	0.5855	0.8525	0.8469	0.0550	0.7140	0.9310
nitrazepam	0.8895	0.8706	0.8584	0.6700	0.7254	0.9188	0.8691	0.1420	0.9030	0.9500
oxazepam	0.9186	0.8081	0.8455	0.7877	0.7733	0.9296	0.9149	0.2520	0.8250	0.9280
diazepam	0.9390	0.8706	0.8798	0.8278	0.8225	0.9513	0.9241	0.2720	0.8770	0.9390

CHCl3 i-PrOH Me2O Estimated Arf min

0	1	2	3	4	5	6	7	8	9	
metazepam	0.8407	0.6656	0.8790	0.3895	0.4114	0.8321	0.7715	0.1033	0.6266	0.7973
napoton	0.8620	0.7711	0.9058	0.4404	0.6218	0.8449	0.8130	0.1176	0.7241	0.8531
nitrazepam	0.8977	0.8865	0.9409	0.5339	0.7569	0.9317	0.8394	0.2161	0.8920	0.9017
oxazepam	0.9236	0.8561	0.9395	0.6228	0.8048	0.9482	0.8739	0.3430	0.7969	0.8741
diazepam	0.9529	0.9035	0.9570	0.6536	0.8585	0.9749	0.8897	0.3660	0.8550	0.8922

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Program functions

Data

solvents CHCl3 i-PrOH Me2O

experiment 0 1 2 3 4 5 6 7 8 9

compound\_pair (metazepam, metazepam), (metazepam, napoton), (metazepam, nitrazepam), (metazepam, oxazepam), (metazepam, diazepam), (napoton, napoton), (napoton, nitrazepam), (napoton, oxazepam), (nitrazepam, nitrazepam), (nitrazepam, oxazepam), (diazepam, diazepam), (diazepam, oxazepam)

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