This is the post-print version of a paper published in Journal of Chromatography A

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Fast Estimation of Adsorption Isotherm Parameters in Gradient Elution Preparative Liquid Chromatography II: The Competitive Case

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Abstract

Experimental competitive adsorption isotherms were successfully determined directly from overloaded

elution profiles in gradient elution mode using an extended inverse method. This approach differs from

the existing methods in one important aspect – no isocratic experiments are necessary which makes it

possible to study adsorption of substances whose retention factors vary strongly with the mobile-phase

composition. The approach was verified with simulated binary data and with experimental data from

gradient separations of a cyclohexanone/cycloheptanone mixture. For the synthetic data, the original

adsorption isotherm parameters were found using a two-step estimation procedure. In the first step

20 analytical peaks were used to estimate the "analytical" part of the Langmuir equation and in the second

21 step the association equilibrium parameters were estimated from two simulated overloaded elution

22 profiles.

23 For the experimental data, a three-step approach was used. The two first steps were used to reduce the

24 calculation time so that parameter estimation could be performed on an ordinary computer. In the first

25 step, analytical peaks were used to estimate the "analytical" part of the bi-Langmuir equation. In the

26 second step, initial guesses for all other parameters were determined separately for each solute using

27 the faster Rouchon algorithm. In the final and third step, the more accurate orthogonal collocation on

28 finite elements algorithm, was used to fine-tune the isotherm parameters. The model could accurately

29 predict the shape of overloaded elution profiles. The shape of the adsorption isotherms agreed well with

30 those determined with the standard isocratic method, although the numerical values were not the same.

31 The extended inverse method is well suited for process optimization where few experiments and

32 accurate predictions are important.

33 *Keywords:* RPLC; Gradient elution; Inverse method; Competitive adsorption isotherm

1 Introduction

35 Gradient elution is extremely important in analytical liquid chromatography but is used to a lesser extent

36 in preparative liquid chromatography. Of all studies published based on the theory for band migration

37 and adsorption in overloaded chromatography [1] only a few concerns non-isocratic gradient conditions.

38 But these few studies shows the great potential for calculating optimal conditions for preparative

39 gradient chromatography both in batch separation [2–8] and in simulated moving bed [9,10].

40   A necessary step in numeric process optimization is estimation of the competitive adsorption isotherms

41   [1]. Although well-developed optimization routines exist for gradient elution [5–8], it is tedious to

42   determine the nonlinear adsorption isotherms in this mode, especially when the feed is a mixture of two

43   or more solutes [8,11–18]. In gradient elution the adsorption isotherms are first estimated using isocratic

44   experiments for several different fractions of modifier in the eluent, here the adsorption isotherm model

45   is assumed to be independent of the fraction of modifier used and the modifier only affects the

46   adsorption isotherm parameters. Then a function that describes the variation of the adsorption isotherm

47   parameters with the modifier fraction must be determined. This "classical approach", where the

48   adsorption isotherms are determined with isocratic experiments on different mobile-phase plateaus, can

49   lead to extreme retention times for some mobile-phase compositions and therefore it might even be

50   impossible to determine all necessary adsorption data.

51   The inverse method is a fast method for the estimation of competitive adsorption isotherms [19–22], but

52   to our knowledge it has only been used for isocratic elution. In order to use the "classical" version of the

53   inverse method in gradient elution mode one would need to perform fraction analysis and peak

54   deconvolution [23]. In a previous fundamental "proof-of concept" study, we developed and verified an

55   approach that used the inverse method to obtain adsorption data directly in the gradient mode [24] for

56   the single component case. The approach, denoted the extended inverse method, eliminates the need

57   for isocratic experiments. The approach was successfully used to estimate single component adsorption

58   isotherms directly from overloaded gradient elution profiles. When compared to the isocratic

59   perturbation peak method the adsorption isotherm parameters were not the same for the two methods,

60   but the shape of the adsorption isotherm was. Both methods gave excellent predictions of elution

61   profiles.

62 In all separations of practical use we have more than one component. Although the extended inverse

63 method may not always give physically correct adsorption parameters it still have a number of important

64 applications such as determination of adsorption isotherms for process optimization or when the

65 classical methods fail due to extreme retention times at low modifier fractions or very large difference

66 between components retention factors. Therefore, in this more "process-orientated" study, the aim is to

67 extend our previous single component approach [24] to determine also competitive adsorption

68 isotherms in gradient mode. Since the competitive case is more computer-time consuming as compared

69 to the simpler one-component case, we also aim to develop strategies to speed up the calculations to

70 allow the use of a regular PC. The approach was verified using gradient data from both simulated and

71 real, experimental competitive binary separations. Finally, we will also compare the column models

72 ability to predict gradient elution profiles if we use only isocratic (classical) or only gradient (our new

73 method) experimental chromatograms in the invers method calculations.

74 ## 2 Theory

75 The calculation of elution profiles was done using the equilibrium-dispersive model that was solved with

76 the orthogonal collocation on finite elements method (OCFE). The optimization algorithm used in the

77 inverse method was a modified least squares Marquardt algorithm. For more information regarding the

78 numerical calculations see our previous paper [24].

79 In reversed phase chromatography the competitive Langmuir adsorption isotherm is the most commonly

80 used model for multi-component gradient elution. E.g. it has been used to model mixtures of small

81 molecules [7,8] and a mixture of two chemotactic peptides [11]. When the modifier dependence of the

82 adsorption parameters are described by linear solvent strength (LSS) theory [25,26], the two component

83 Langmuir model for component i can be written,

84
$$q_i\left(C_1,C_2,\phi\right) = \frac{a_i e^{-S_{a,i}\phi} C_i}{1 + b_1 e^{-S_{b,1}\phi} C_1 + b_2 e^{-S_{b,2}\phi} C_2},$$
(1)

where q and C are the stationary and mobile phase concentration, ϕ is the volume fraction of organic modifier in the mobile phase and S_* are empirical adsorption parameters that are determined experimentally. Eq. (1) was used here to simulate test data for our inverse method approach.

When the adsorption energy distribution is heterogeneous the two site competitive bi-Langmuir adsorption isotherm can be used instead,

$$q_i\left(C_1,C_2\right) = \frac{a_{I,i} C_i}{1 + b_{I,1} C_1 + b_{I,2} C_2} + \frac{a_{II,i} C_i}{1 + b_{II,1} C_1 + b_{II,2} C_2}.$$
(2a)

If the same relationship as in Eq. (1) is used to describe the modifier dependence of the adsorption parameters the two-component bi-Langmuir model can be written,

$$q_i\left(C_1,C_2,\phi\right) = \frac{a_{I,i} e^{-S_{aI,i}\phi} C_i}{1 + b_{I,1} e^{-S_{bI,1}\phi} C_1 + b_{I,2} e^{-S_{bI,2}\phi} C_2} + \frac{a_{II,i} e^{-S_{aII,i}\phi} C_i}{1 + b_{II,1} e^{-S_{bII,1}\phi} C_1 + b_{II,2} e^{-S_{bII,2}\phi} C_2},$$
(2b)

where subscripts I and II refers to two adsorption sites with different adsorption energy. Eq. (2b) was successfully used in this study to model the adsorption of a cyclohexanone/cycloheptanone mixture on a C18-column in gradient elution with methanol as the organic modifier.

3 Experimental

3.1 Chemicals and Materials

A 150 × 4.6 mm Kromasil column (AkzoNobel Eka, Bohus, Sweden) packed with C18-bonded porous silica, with an average particle diameter of 5 μm, was used. The experiments were performed on an

5

101   Agilent 1200 system (Palo Alto, CA, USA) consisting of a binary pump system, an auto-sampler with a 900

102   µL injection loop, a diode array UV detector and a column thermostat. The column temperature was held

103   constant at 22°C with the thermostat and the flow rate was 1.0 mL/min for all experiments.

104   Cyclohexanone (≥99.5%) and cycloheptanone (≥99%) from Sigma-Aldrich (Steinheim, Germany), was

105   used as solutes while dichloromethane (≥99.5%) from VWR International (Paris, France) and 2-propanol

106   (HPLC grade) from Fisher Scientific (Loughborough, UK) were used in the pycnometry measurements.

107   The mobile phase consisted of HPLC grade methanol from Fisher Scientific (Loughborough, UK) and de-

108   ionized water, with conductivity 18.2 MΩ cm, delivered from a Milli-Q Plus 185 water purification system

109   from Millipore (Merck Millipore, MA, USA).

110   3.2 Experimental Data

111   Calibration curves for cyclohexanone and cycloheptanone were recorded at 280 nm for seven mobile-

112   phase compositions. The calibration curves were linear (R^2 ≥ 0.9996 up to 0.2 M) for both substances at

113   all mobile-phase compositions, but the slopes varied somewhat with the methanol fraction. Therefore

114   the calibration curve that was closest to the methanol fraction in the eluent at the time the elution

115   profile was recorded was used to convert that elution profile to concentration. The total area under the

116   peaks in the elution profiles was adjusted so it matched the injected amount of solute. The column hold-

117   up volume was measured with pycnometry following the procedure outlined in [27]. This resulted in a

118   column hold-up volume of 1.38 mL.

119   The apparent average plate number was measured to 5000 for cyclohexanone and previously we

120   measured the average plate number for cycloheptanone to 2000 [24]. In the modeling of these solutes

121   we will use 2 000 plates for both solutes, the reason is to reduce the calculations time. It is easy to use

122   different efficiency for the solutes, but it would cost time and would not affect the end result. Previously

123   we have studied isocratic separations of compounds with different efficiency between the compounds

124   [28]. In that study the elution profiles were modeled using both different and identical efficiency for the

125   compounds. We found no difference between the models ability to predict elution profiles as long as the

126   least efficient solute had more than 1 000 plates. We strongly believe that the introduced errors due to

127   this simplification could be negligible.

128   The injection profile is sensitive to flow rate, injection volume and solute/eluent composition [29]

129   therefore the experimental injection profile was recorded and used in the calculations. The injection

130   profile of the overloaded injections at flow rate 1.0 mL/min was recorded with the column replaced with

131   a zero-volume connector and was fitted to an empirical equation; see Eq. (2) in [24]. The area under the

132   injection profile was then adjusted for the amount of injected sample. Observe that we do not use the

133   true injection profiles entering the column inlet. Our injection profile includes both column-inlet and

134   column-outlet effects which cannot be distinguished. However, using this experimental injection profiles

135   will be more accurate than using the rectangular injection profiles that is frequently used. We would also

136   like to stress that in most other studies where the experimental injection profile is accounted for, the

137   column-inlet and column-outlet effects are not separated [21,29–31]. Finally, the inverse method

138   incorporates such errors in the isotherm parameters, which are empirical anyway.

139   Overloaded elution profiles were recorded by injecting 400 µL of a sample containing cyclohexanone and

140   cycloheptanone in equal concentrations. Four different sample concentrations were used; 0.1, 0.2, 0.3

141   and 0.4 M. Analytical peaks for the two solutes were also recorded by injecting 5 µL of 10 mM samples.

142   This was done for seven modifier plateaus; 24, 30, 35, 40, 46, 51 and 56% methanol and four linear

143   gradients. The gradients ran from 24% to 56% methanol and the slopes of the gradients were 1, 2, 3 and

7

144   4%/min. Before each gradient run, the column was equilibrated with the methanol fraction at the

145   beginning of the gradient. The time it took for the gradient to reach the column inlet after the injection

146   was initialized was 86 s. At least two replicates were done for all injections.

4 Results and Discussion

148   To verify our extended inverse method both simulated and experimental data was used. First, as a proof

149   of concept, the competitive adsorption isotherm was determined from computer generated gradient

150   elution profiles. Then, as a reference, the competitive adsorption isotherm for cyclohexanone and

151   cycloheptanone were determined using only isocratic experiments at different modifier fractions with

152   the standard inverse method. Thereafter competitive adsorption isotherm was determined for the

153   experimental case again, but now using the extended inverse method directly from only gradient elution

154   profiles. Finally, the results from the isocratic and gradient approach were compared.

4.1 Verification Using Simulated Data

156   Here we assumed a system with a 150 × 4.6 mm column, with hold-up time 1.5 min and with 1 000

157   theoretical plates to simulate elution profiles. The flow rate was 1 mL/min and 400 μL samples, with

158   concentration 0.4 M, were introduced using rectangular injection profiles. Linear gradients, with slopes 1

159   and 4 %/min that started 0.5 min after the beginning of the injection, was employed and the modifier

160   fraction was varied from 30% to 60%.

161   The competitive Langmuir adsorption isotherm, Eq. (1), was used with the adsorption isotherm

162   parameters presented in Table 1. Two elution profiles with sample concentration 0.4 M and gradient

163   slope 1 and 4%/min was used in the inverse method.

164   First we considered the case where the saturation capacity is assumed to be independent of the modifier

165   concentration. For Eq. (1) the saturation capacity $q_{s,i}$ for component i is,

166
$$q_{s,i} = \lim_{C_i \to \infty, C_{j \neq i} = 0} q_i = \frac{a_i e^{-S_{a,i}\phi}}{b_i e^{-S_{b,i}\phi}},$$
(3)

167   and for this to be independent of the modifier concentration one must have that $S_{a,i} = S_{b,i}$. When this is

168   assumed the six adsorption isotherm parameters could be estimated simultaneously with success, i.e.,

169   the original parameters could be obtained with at least three decimals accuracy. Then we considered the

170   case where the constraint on the saturation capacity was removed and all eight parameters were

171   estimated simultaneously. The optimization algorithm then failed to converge to the correct parameters,

172   see Fig. 1 and Table 1 "Simultaneously". This could either be due to that it gets stuck in local minima or

173   due to that the algorithm failed to estimate the gradient of the objective function with sufficient

174   accuracy.

175   To prevent this, one could choose another optimization algorithm, e.g. simulating annealing [32] that

176   does not use derivatives and can "escape" from local minima. If the problem is due to insufficient

177   accuracy of the gradient one could instead try to increase the calculation accuracy in the ODE solver [33]

178   which solves the set of ordinary differential equations obtained after discretization of PDEs in the OCFE

179   method. Both these alternatives would seriously increase the calculation time and make the inverse

180   method impractical to use. As a work around, the parameter estimation can be done in two steps:

181   1. First the linear version of Eq. (1), with $b_i = 0$, is used to estimate the four a-parameters from

182   analytical injections for the two gradients, the original parameters can then obtained.

9

183   2.   In the second step the four *b*-parameters can successfully be estimated from the two overloaded

184   profiles described above, see Table 1. Here it was also noted that at least two elution profiles

185   obtained with different gradients were needed for successful estimations the adsorption isotherm

186   parameters in gradient mode.

187   **4.2 Determination of Adsorption Parameters from Experimental Isocratic Data**

188   The standard, isocratic inverse method [19] was used to estimate the competitive adsorption isotherm

189   parameters for the mixture of cyclohexanone and cycloheptanone on seven eluents with different

190   methanol fractions. To test if the LSS theory could be used to describe the retention factor's methanol

191   dependency, analytical injections were conducted at eluents with different modifier content. The ln(k)

192   versus ϕ plot for the analytical peaks, was linear with $R^2 = 0.9985$ and $R^2 = 0.9989$ for cyclohexanone and

193   cycloheptanone, respectively. This indicates that LSS theory could be used in this case.

194   For each of the seven investigated methanol fractions, four elution profiles with different concentrations

195   were used to estimate the bi-Langmuir adsorption isotherm, Eq. (2a). The bi-Langmuir model fitted the

196   isocratic data well with an average overlap of 93%. Because the LSS theory described the methanol

197   dependence of the retention factors accurately it was also used to describe the methanol dependence of

198   the adsorption isotherms parameters, see Fig. 2. The LSS model parameters are presented in Table 2

199   along with the correlation coefficients. The values for the second adsorption site were omitted for

200   cycloheptanone at the methanol fractions 0.24 and 0.40 because the association equilibrium constant

201   was close to zero (1.3×10^{-4} and 2.1×10^{-6}, respectively) at these methanol fractions. The LSS model

202   described the methanol dependence of both solutes well.

203 Finally, the determined adsorption isotherms were used to predict overloaded gradient elution profiles,

204 see Fig. 3. The agreement between experimental and calculated elution profiles was good with an

205 average area overlap equal to 85%, which is of similar quality as other previously published results for

206 binary gradient elution [7,8,11].

207 **4.3 Determination of Adsorption Parameters from Experimental Gradient Data**

208 Here experimental gradient elution data was used to estimate the competitive adsorption parameters in

209 Eq. (2b) with the extended inverse method. A simultaneous estimation of all 16 parameters was not

210 possible if the initial values were badly chosen due to a very long calculation time. Also, as in Section 4.1,

211 the optimization algorithm might not converge to the correct, global, solution. To prevent this, and

212 making it possible to conduct the calculations on an ordinary computer, the parameters were estimated

213 using a three step approach:

214 1. In the first step, the "analytical" version of Eq. (2b), i.e., with $b_I = b_{II} = 0$, was used separately for

215   cyclohexanone and cycloheptanone to get initial guesses for a_I, S_{aI}, a_{II} and S_{aII}. This was done by

216   minimizing the difference between the calculated and measured retention times of analytical peaks

217   in gradient elution.

218 2. In the second step, initial guesses for b_I, S_{bI}, b_{II} and S_{bII} was determined separately for the two solutes

219   by using the faster [1], but less accurate [34], Rouchon algorithm to solve the equilibrium-dispersive

220   model. Two experimental profiles were used: the one with the steepest slope and the highest

221   sample concentration (4%/min and 0.4 M), and the one with the lowest slope and sample

222   concentration (1%/min and 0.1 M).

11

223   3.   In the third and final step, all 16 parameters in Eq. (2b) were estimated from experimental, binary

224   elution profiles obtained in gradient mode with the OCFE method with the same two experimental

225   profiles as in step 2.

226   The estimated adsorption isotherm parameters are presented in Table 2. Using these parameters,

227   gradient elution profiles when injecting 400 µL of 0.3 M sample were predicted and compared to

228   experiments for different gradients, see Fig. 4. The agreement between experimental and predicted

229   elution profiles was very good for all gradients with an average overlap of 88%. To further test the

230   models ability to predict elution profiles predictions for all overloaded gradient experiments presented in

231   Section 3.2 were done. Generally, better model predictions were found for the high concentration

232   samples, but no clear difference between gradient slopes were observed. The choice of elution profiles

233   in the third estimation step was found to be important for the predictive power of the estimated

234   adsorption isotherm parameters. If the highest concentration for slope 1 and 4%/min was used in the

235   inverse method instead, the predicted profiles at concentrations below 0.2 M became markedly worse,

236   especially for cyclohexanone.

237   ### 4.4. Comparison between the Isocratic and Gradient Approach

238   Comparing the estimated adsorption isotherm parameters from the two approaches presented in Table

239   2, we can see that they differ a lot. This was also the case for the single component case as we reported

240   earlier [24]. One reason for the difference between the isocratic and gradient approach is that the exact

241   shape of the gradient is not known and even if no adsorption of the modifier exists, some dispersion is

242   present due to the chromatographic system. The gradient shape was recorded without the column

243   attached and the beginning and the end of the gradient was indeed somewhat smoothened. There are

244   also more effects associated in the chromatographic system which need to be accounted for in gradient

245   elution compared to isocratic elution, e.g. potential variations in the column hold-up time during the run,

246   when the gradient reaches the column and potential deformation of the gradient. If the classical

247   approach is used to estimate the adsorption parameters, all these effects must be measured accurately

248   [17]. The extended inverse method, on the other hand, will incorporate any errors from such sources in

249   the model by adjusting the adsorption isotherm parameters. This leads to an empirical model which can

250   be used without as much experimental work.

251   The shape of the competitive adsorption isotherm, with parameters from the two approaches, is plotted

252   at three different modifier plateaus (24%, 40% and 56% methanol) for a 1:1 ratio of cyclohexanone and

253   cycloheptanone in Fig. 5. There is very good agreement between the two adsorption isotherms at 40%

254   methanol in the eluent; this was also the case for the 30%, 35%, 46% and 51% methanol compositions

255   (not shown) for both solutes. The adsorption isotherms differ more for the highest (56%) and lowest

256   (24%) methanol plateaus. It is logical that differences between the two methods can be seen at the

257   highest and lowest modifier plateaus. In gradient elution, the highest methanol fraction is only reached

258   when the solute is close to the column outlet, and only for the two steepest gradient slopes, while the

259   lowest methanol fraction only exists during the injection of the sample.

260   The ability to predict elution profiles are slightly better for the extended inverse method (88% versus

261   85% area overlap) which is to be expected then we uses gradient experiments to determine the

262   adsorption isotherms.

5 Conclusions

We have successfully showed that the extended inverse method could estimate competitive, nonlinear adsorption isotherms directly from overloaded binary elution profiles obtained in gradient elution mode and these estimated adsorption isotherms can be used to predict gradient elution separations.

The approach was first verified with a set of simulated data for the two-component competitive Langmuir adsorption isotherm where LSS theory described the modifier dependence of the adsorption isotherm parameters. The original parameters could be found with a two-step estimation procedure: First, analytical peaks were used to estimate the "analytical" version of the Langmuir equation. Then, from two overloaded elution profiles, the association equilibrium parameters were estimated.

The approach was then verified using experimental gradient elution data for a mixture of cyclohexanone and cycloheptanone. Here a three-step inverse method was used to estimate the parameters in the competitive two-component bi-Langmuir adsorption isotherm. First, analytical peaks were used to estimate the "analytical" part of the bi-Langmuir equation. Then, initial guesses for all other parameters were determined separately for each solute using the faster Rouchon algorithm. Finally, the more accurate OCFE algorithm was used to fine tune the isotherm parameters. The resulting adsorption isotherm was compared with the adsorption isotherms determined using the classical isocratic approach. The adsorption isotherms parameters from the two approaches did not agree, however, the shapes of the adsorption isotherm are similar. The larges difference could be found at the highest and lowest methanol fractions. Gradient elution profiles are also better predicted if adsorption isotherm parameters from the extended inverse method are used instead of ones from the classical isocratic method. If the results here are compared to those in the companion paper [24], we see that the same conclusions can be drawn when comparing the extended inverse method with the classical isocratic approach; that the

285   adsorption parameters should be treated as empirical. Also, a multistep estimation procedure is needed

286   when two or more components are considered.

287   The three main advantages with our extended inverse method are,

288   1. No isocratic experiments are needed, which makes it possible to determine adsorption isotherms in

289   cases where isocratic experiments are not feasible.

290   2. The amount of experimental work is significantly reduced compared to the classical approach.

291   3. The model is not sensitive for errors in system parameters such as column hold-up time, gradient

292   starting time and gradient shape.

293   One challenge encountered with the extended inverse method is the risk that the optimization routine

294   not converges to the correct global solution. The problem was solved by estimating the adsorption

295   parameters in several steps, which also solved the other problem – the long calculation times when bad

296   starting guesses were chosen for the adsorption isotherm parameters. With this approach calculations

297   could be conducted on an ordinary computer. However, it remains to be investigated if there is an

298   optimization algorithm which is better suited for solving this specific optimization problem.

299   What also remains to be investigated are how the extended inverse method performs using other

300   adsorption isotherm models, in principle the step-approach employed here should work. However,

301   theoretical knowledge of how the parameters change with the modifier fraction does not exist for

302   adsorption models like Tóth or Moreau. But the bi-Langmuir model, together with the LSS model,

303   provides a flexible model that should be able to describe a lot of different adsorption isotherms, at least

304   approximately.

305   In our opinion the extended inverse method is very suitable to be used as a tool in process optimization

306   were the models ability to predict elution profiles is essential and few experiments are preferred. For

307   more fundamental adsorption studies, classical, isocratic methods, e.g. frontal analysis or the

308   perturbation peak method, are still recommended.

Acknowledgements

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Figure captions

Fig. 1: Calculated elution profiles with the original (solid lines) and "estimated simultaneously" (dashed lines) adsorption isotherm parameters for the synthetic system.

Fig. 2: Methanol dependence of the competitive bi-Langmuir adsorption constants for cyclohexanone and cycloheptanone. The symbols are the parameter values found when fitting the bi-Langmuir model with the inverse method on modifier plateaus, open squares denote site I and filled circles denote site II. Solid lines represent the best fit to LSS theory.

Fig. 3: Comparison between predicted, from isocratic experiments, (solid lines) and experimental (dashed lines) elution profiles at different gradient slopes for a mixture of cyclohexanone (first peak) and cycloheptanone (second peak). Sample concentration is 0.3 M and injection volume is 400 µL.

Fig. 4: Comparison between predicted, from gradient experiments, (solid lines) and experimental (dashed lines) elution profiles at different gradient slopes for a mixture of cyclohexanone (first peak) and cycloheptanone (second peak). Sample concentration is 0.3 M and injection volume is 400 µL.

Fig. 5: Comparison between the competitive adsorption isotherms with parameters from isocratic experiments (symbols and dashed lines) and the parameters from gradient experiments (solid lines) at three different methanol-water mobile phase compositions. The concentration ratio of cyclohexanone and cycloheptanone is 1:1.

Table 1: True and estimated adsorption parameters, with 95% confidence intervals, from simulated elution profiles. The adsorption parameters where estimated either simultaneously or by a two-step approach.

	a_1	$S_{a,1}$	b_1	$S_{b,1}$	a_2	$S_{a,2}$	b_2	$S_{b,2}$
True	150	7.5	100	7	40	6.5	30	6
Simultaneously	193.7	8.142	204.9	8.808	65.60	7.912	91.18	9.305
2-step approach	150.0	7.500	99.97	6.999	40.00	6.500	29.95	5.995

Table 2: Estimated adsorption parameters for cyclohexanone (C6) and cycloheptanone (C7) from real experimental data with the inverse method from both isocratic and gradient experiments.

Solute	a_I	S_{aI}	b_I [M^{-1}]	S_{bI}	a_{II}	S_{aII}	b_{II} [M^{-1}]	S_{bII}
C6 – Isocratic[1]	12.38	5.012	4.875	3.974	38.36	7.811	795.2	11.85
C6 – Gradient	0.2877	0.4233	4.00	4.594	41.22	6.750	27.09	5.725
C7 – Isocratic[2]	33.69	5.814	16.20	5.390	120.8	8.438	436.6	8.066
C7 – Gradient	4.628	0.6646	73.77	2.896	112.2	8.245	170.6	10.17

[1] With R^2 = 0.966, 0.986, 0.944 and 0.744.

[2] With R^2 = 0.975, 0.978, 0.980 and 0.938.