Comparison of chromatographic stationary phases using a Bayesian-based multilevel model

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ABSTRACT: Herein, we used a Bayesian multilevel model of chromatographic retention to compare five reversed-phase high-performance liquid chromatography stationary phases: XBridge Shield RP18, XTerra MS C18, XBridge Phenyl, XBridge C8, and Xterra MS C8. For this, we used a large dataset of retention times collected using chromatographic techniques coupled with mass spectrometry. The experiments were conducted in a gradient mode for an initial mixture of 300 small analytes for a wide range of pH values in methanol and acetonitrile at two temperatures and for three gradient durations. Our analysis was based on a mechanistic model derived from the principles and fundamentals of liquid chromatography and utilized previously reported chromatographic parameters. The data and model were used to characterize the between-column differences in the chromatographic parameters of neutral, acidic, and basic analytes. The analysis provides an interpretable summary of stationary-phase properties that can be used in decision-making, i.e., finding the best chromatographic conditions using limited experimental data. The proposed approach is an interesting alternative to the existing ones used to compare chromatographic stationary phases.

The selection of chromatographic stationary phases is an important aspect of an analytical study. Over the years, numerous methods and approaches have been proposed to classify, select, and characterize chromatographic stationary phases.1–4 All the current methods use various sets of probe analytes and a set of chromatographic conditions to estimate the most important chromatographic characteristics, e.g., column efficiency, hydrophobicity, silanol activity, ion-exchange capacity, and steric selectivity, as well as/or to calculate various similarly metrics. Usually, stationary phases are compared based on parameters/similarity metrics derived from simple empirical/statistical models and, as such, do not necessarily generalize to other analytes, chromatographic conditions, and stationary phases. Moreover, obtaining very detailed physical (and thermodynamic) descriptions of chromatographic systems is possible using enthalpies and entropies of transfer or absorption isotherms. Nevertheless, such methods requires datasets collated based on extensive sets of carefully controlled experiments, which are possible only for a few selected analytes/problems.5

The chromatographic stationary phases can also be compared based on retention time data collected for a group of structurally heterogenous compounds. Nowadays, it is possible to collect such large datasets using chromatographic techniques coupled with mass spectrometry. Because such datasets carry considerable information about chromatographic retention, they can be used to perform detailed multidimensional characterizations of chromatographic stationary phases, build a mechanistic model, and predict retention times based on various number of preliminary experiments (e.g., to predict the retention time for a set of analytes for several measured data collected using a different stationary phase). In principle, the prediction results obtained using a model based on such data can be generalized to other analytes, stationary phases, and chromatographic conditions. The retention time predictions in liquid chromatography that are accurate or with well calibrated uncertainties are required for rapid column screening, computer-assisted method development, and method transfer.6

In this study, we applied the previously developed Bayesian multilevel framework7–10 to characterize chromatographic gradient retention time datasets collected using a multicomponent mixture of analytes, five stationary phases (XBridge Shield RP18, XTerra MS C18, XBridge Phenyl, XBridge C8, and Xterra MS C8), and a wide range of chromatographic conditions (pH, organic modifier, temperature, and gradient program). The general idea was to develop a mechanistic model of chromatographic retention that jointly characterized the retention of available data, enabled estimation of the stationary-phase (column) effects on the chromatographic parameters of acids, bases, and neutral analytes, quantified various sources of variations, and characterized the inherent uncertainties. The usefulness of the developed model in decision-making was illustrated by searching chromatographic conditions that led to the desired separation based on different preliminary data, e.g., prediction of retention on the tested columns for given XBridge Shield RP18 data. This part was based on the statistical decision theory.

# EXPERIMENTAL SECTION

## Data

The data were collected by performing 84 different liquid chromatography experiments using an initial mixture of 300 analytes. In the experiments, the gradient duration (30, 90, and 270 min), pH of the mobile phase (from 2.5 to 10.5), type of organic modifier (methanol (MeOH) or acetonitrile (ACN)), and column temperature (25°C and 35°C) were varied.

Briefly, the liquid chromatography experiments were carried out with an Agilent Technologies 1260 Infinity system (Agilent Technologies, Waldbronn, Germany) and a 6224 time-of-flight (TOF) mass spectrometer with a dual electrospray ionization source (Dual ESI) in the positive polarity mode using XBridge Shield RP18, XTerra MS C18, XBridge Phenyl, XBridge C8, and Xterra MS C8 Waters Ltd., Milford, MA, USA, 3 mm × 50 mm, 2.5 μm). The extra column volume (*Ve*) and system dwell volume (*Vd*) were 0.020 and 1.05 mL, respectively. The column hold-up volumes (*V0*) were 0.266, 0.271, 0.271, 0.276, and 0.284 mL, and the flow rate (*F*) was 0.5 mL/min.

Ammonium bicarbonate, ammonium acetate, and ammonium formate were selected as buffers to control the pH of the mobile phase during the chromatographic separation. The pH of the buffers (nominal aqueous pH) was adjusted to the desired levels (ammonium formate: 2.5, 3.3, 4.1, 8.9, and 9.7; ammonium acetate: 4.9 and 5.8; and ammonium bicarbonate: 6.8 and 10.5) via addition of formic acid, acetic acid, and ammonia, respectively. The pH was measured at 25°C and 35°C using an S220 pH meter (Mettler Toledo, Greifensee, Switzerland) with an InLab® Routine Pro ISM electrode after mixing an organic modifier with the buffer solution.

The MassHunter Profinder B.08.00 (Agilent Technologies, Waldbronn, Germany) system was used to identify all the matches per formula using “Batch Targeted Feature Extraction” (containing 300 predefined masses for each analyte included in the mixture). The data for analysis were restricted to analytes that had “IdentificationScores” higher than 95%, that were present on at least half of the chromatograms (total and per column), and that had less than two dissociation steps in a pH range from 2 to 11.

The functional groups and structural elements were determined using Checkmol (version 0.5b N. Haider, University of Vienna, 2003–2018).11 The lipophilicity (*log P*) and dissociation constant (*pKalit*) were calculated using the ACD/Labs program12 based on the structures of the analytes generated from SMILES strings.

More details on the data extraction process are reported elsewhere.7 The raw data used for the selected analytes are shown in Figure S1.

## Structural Model

The details of the model are provided in the Supplementary Materials. Briefly, a standard chromatographic model was employed in this study.13,14 For each analyte, the effect of the pH was described using a function that linked the isocratic retention factor and *pH* with *R* dissociation steps and *R + 1* forms15:

(1)

where *r* represents thedissociation step, denotes the *rth* dissociation constant, andrepresents the retention factor of a particular form of the analyte under a given chromatographic condition. Furthermore, we assumed that depends on the organic modifier content, pH, and temperature based on the following equation:

(2)

where represents the base-10 logarithm of the retention factors extrapolated to 0% of organic modifier content at 25°C for a mobile phase with a pH of 7 for the neutral and dissociated forms of the analyte; and denote slopes in the Neue equation; denotes the change in due to an increase in the temperature by 10°C; and *apHr* denotes the pH effects for cations and anions. In this parametrization of the Neue equation, the *S1* parameter reflects the difference between the logarithm of the retention factors of the 0% and 100% organic modifier contents.

Furthermore, a linear relationship between the *pKa* values and organic modifier content is assumed as follows:

(3)

where denotes the dissociation constants of an analyte under given chromatographic conditions, denotes aqueous *pKa*, and denotes the slope due to changes in the organic modifier.

## Measurement-Error Model

The observed retention factors (*tRobs,z*) were modeled using the following equation:

(4)

where *z* is the *zth* measurement, and *student\_t* denotes the student’s t-distribution with the mean given by the predicted retention time *tR,z*, scale *σ* (analyte and column specific), and normality parameter . The retention time *tR,z* for a certain organic modifier gradient was calculated using the following integral equation:

(5)

where *t0*denotes the column hold-up (dead) time, *te* is the extra column time, and is the instantaneous isocratic retention factor corresponding to the mobile phase composition at time *t* at the column inlet for a particular observation. This integral equation was numerically solved using steps 4 and 10 for methanol and acetonitrile gradients proposed by Nikitas et al.13

## Analyte-Level Model

The parameters of each analyte were assumed to be different to account for the between-analyte variability (BAV). The following relationship was used in this analysis:

(6)

where *Ri* denotes an individual value of a parameter *R*, is the mean value for an analyte with *logP =* 2.2, is the regression coefficient, anddenotes the standard deviation. For the correlated parameter, a multivariate normal distribution was used.The BAV was assigned for *logkwN* and *S1mN* (*S1* in MeOH) for the neural (N) form of the analytes (these parameters were correlated (ρ) and depended on *logP* (*β*)). Further, the effects of ACN on *S1* (*dS1N*), that of temperature on logkw (*dlogkT*), that of dissociation on *logkw*, *S1m*, and *dS1* (*dlogkw*, *dS1m*, *ddS1)* separately for acids (A) and bases (B)), and that of the column on these parameters (*clogkw*, *cS1m*, *cβ*, *cdS1*, *cdlogkT*, *cdlogkw*, *cdS1m*, *cddS1*) were used to characterize the impact of the chromatographic conditions on the model parameters. Further, the *clogkw* parameters for the neutral forms of the analytes were found to be correlated (cρ) during the model development process. The pH effects for cations and anions (*apHA* and *apHB*) were assumed to be the same for all the analytes, but different for the columns (*capHA* and *cpHB*). The *S2* parameter was assumed to be the same for the analytes and columns, but different in MeOH and ACN (*logS2m*, and *dlogS2* for ACN effect). Further, the BAV was assigned for the dissociation related parameters: *pKaw* (dependent on literature *pKa* values), parameters of acids and bases in MeOH (, and effect of ACN on (. The *pKa* and parameters were assumed to be independent of the column. The standard deviation in BAV was denoted by *ω* for the parameters of the neutral form of the analyte on XBridge Shield RP18 and by *cω* for the column effects, *ωT*and *cωT* for temperature effects, *κ* and *cκ* for the parameter related to dissociated forms, and *τ* for the parameters related to the dissociation constant.

## Priors

The Bayesian model requires specification of priors that allow incorporation of domain expertise into inferences. In this study, priors were selected based on literature knowledge, as previously discussed.7 The means for the priors describing column effects were assumed 0. The standard deviation values were set to about half of the standard deviations/scales used for the XBridge Shield RP18 parameters. Such priors assume a very weak similarity of the compared stationary phases across various conditions. The exact values are given in Supplementary Materials.

## Bayesian Inference

**Technical.** Multilevel modeling was performed using the Stan/cmdstanr16 software linked with Rstudio17. For the inference, we used eight Markov chains with 500 iterations after 1000 warm up iterations. The *reduce\_sum* function was used to accelerate the calculations by parallelizing the execution of a single Stan chain across multiple cores. Convergence diagnostics were performed using Gelman–Rubin statistics and trace plots, and the results indicated that the model results did not diverge. The R code, data, and Stan code used to analyze the data are publicly available in the GitHub repository (https://github.com/wiczling/columncomparison). The raw data are also accessible through a osf.io repository18–22. The calculations were run on the Tryton computing cluster in the Center of Informatics Tricity Academic Supercomputer and Network.

**Predictions Using a Limited Set of Experiments.** The applicability of the proposed model was illustrated using six analytes with different acidic/basic properties: acridine (monoprotic acid), baclofen (zwitterion: acidic and basic group), hydrocortisone (neutral), pioglitazone (zwitterion: basic and acidic group), quinine (diprotic: two basic groups), and tolbutamide (monoprotic base). In this paper, we present the limited data predictions that correspond to future predictions or retention times based on experimental data collected for the XBridge Shield RP18 column. For this purpose, all the population parameters were fixed to a posteriori estimates. The individual parameters of the six selected analytes were re-estimated solely using limited data. It allowed us to predict retention times or retention factors for the other columns assuming a good understating of the retention in one reference column (specifically XBridge Shield RP18 column).

**Uncertainty chromatogram**. The predictions were summarized as uncertainty chromatograms (posterior distribution of retention times expected for a given set of chromatographed analytes under given conditions).12 Here, we used typical model predictions (without residual variability) for a particular analyte. The uncertainty chromatograms showed the uncertainty of the retention time corresponding to the maximum of each peak on a given chromatogram.

**Decision-making.** The Bayesian optimal decision was sought based on the maximum of expected utility calculated across a wide range of chromatographic conditions. Simulations were performed for *fio* ranging from 0.05 to 0.15 with an increment step of 0.02, *pHo* ranging from 2.5 to 10.5 with an increment step of 0.2, *tg* ranging from 20 to 200 with an increment step of 20, using MeOH and ACN as organic modifiers and for five tested columns at 25°C. The utility function (*U*) was used to calculate the value of each possible chromatogram (posterior predicted retention times). It was defined based on the lowest retention time detected among the analytes (*mintr*), the highest retention time detected among analytes (*maxtr*), and the difference between the retention times of the critical pair of analytes (*res*). This utility was assumed zero if at least one of the analytes had retention higher than 40 or less than 2 min, and if the *res* is less than 2. Otherwise, the utility was linearly related to *maxtr*,favoring shorter runs.

U (7)

The expected utility was plotted for a dense grid of chromatographic conditions yielding the expected utility map. The graph was used to identify chromatographic conditions with high/the highest utilities.

# RESULTS AND DISCUSSION

In this study, we applied a mechanistic model to describe the retention data of small molecules obtained for a wide range of chromatographic conditions and for five chromatographic columns. The model was constructed based on the known fundamentals of gradient chromatography and prior knowledge available in the literature. The methodology allowed us to characterize the stationary-phase properties using the commonly used chromatographic parameters (specifically *logkw*, *S1* and *S2* of the Neue model). Further, the method facilitated drawing of easy interpretations of the estimated effects and application of results in the decision-making step. The model parameters are summarized in Table S1 and Figure S2, and the key parameters are provided in Figures 1–3.

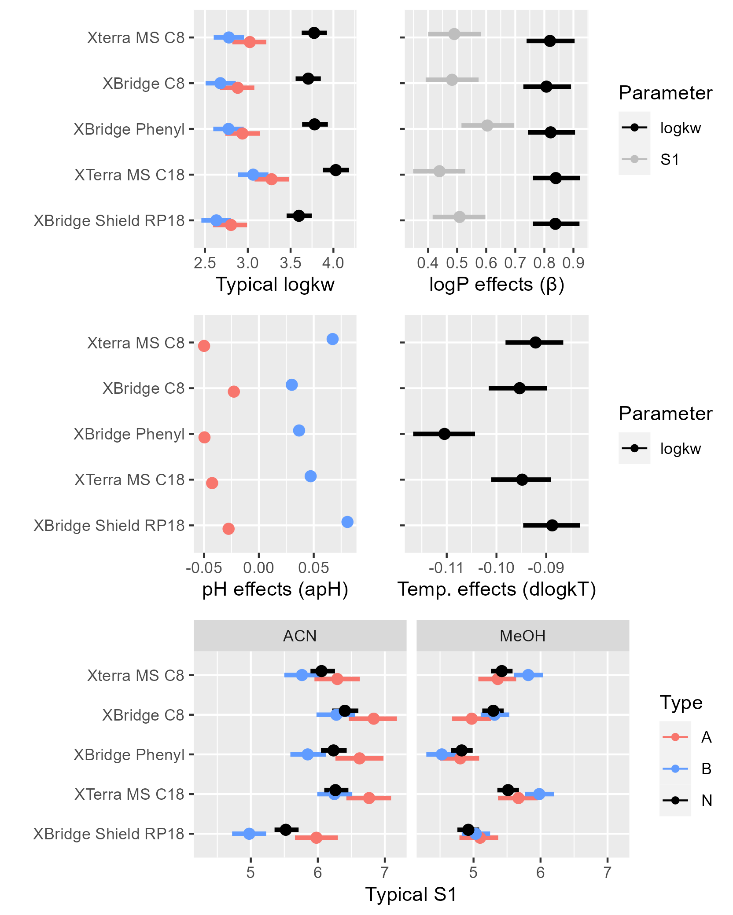


Figure 1. Summary of the marginal posterior distributions of the key population-level parameters that characterize the retention of analytes for all the tested columns.

These parameters provide a concise summary of the effects of various factors on the retention of analytes on the tested columns. Each parameter quantifies the effect of a chromatographic condition/analyte property (pH, organic modifier type and content, temperature, column type, and log P) on the retention of neutral, acidic, and basic forms of an analyte. For simplicity, we will discuss the mean values of the posterior distribution of a particular parameter. The entire posterior distribution is presented graphically in Figures 1-3, Figure 4S and Table S1. The typical *logkw* of a neutral form of an analyte with a log P value of 2.2 (a measure of hydrophobicity) is 3.6 for XBridge Shield RP18 at 25°C and the typical *logkw* values are higher by 0.42, 0.17, 0.10, and 0.17 for the XTerra MS C18, XBridge Phenyl, XBridge C8, and Xterra MS C8 columns, respectively. Compared to XBridge Shield RP18, these values correspond to a typical 2.64, 1.48, 1.26, and 1.48-fold higher retention factors, respectively, for a neutral form of an analyte in neat water eluents. The typical slope (S1) in MeOH is 4.96, and the difference between XBridge Shield RP18 and the other columns is 0.59, −0.12, 0.36, and 0.48, respectively. The BAV for *logkw* and *S1* (*ωlogkw* and *ωS1*) for XBridge Shield RP18 is approximately 0.9 with a high correlation of 0.87, implying the existence of large mutual information of the analyte-specific values of these two parameters. The BAVs for column effects on *logkw* (*cωlogkw*) are small (0.10–0.13). They are also correlated with correlations (*cρ*) ranging from 0.55–0.92 depending on the compared columns. The BAV for column effects on S1 in MeOH (*cωS1m*) are also small (0.02–0.15). Because the BAVs are on a logarithmic scale with base 10, all the standard deviations should be interpreted accordingly to understand the BAV of the retention factor. For example, the BAV of ~0.1 for *clogkw* implies that the predicted effects of the column are of the order of ±0.1, which corresponds to a multiplicative retention factor in water-reach eluents ranging from 10−0.1 = 0.79 to 100.1 = 1.26. High values of BAV for column effects indicate column orthogonality with respect to the retention behavior controlled by a particular parameter.

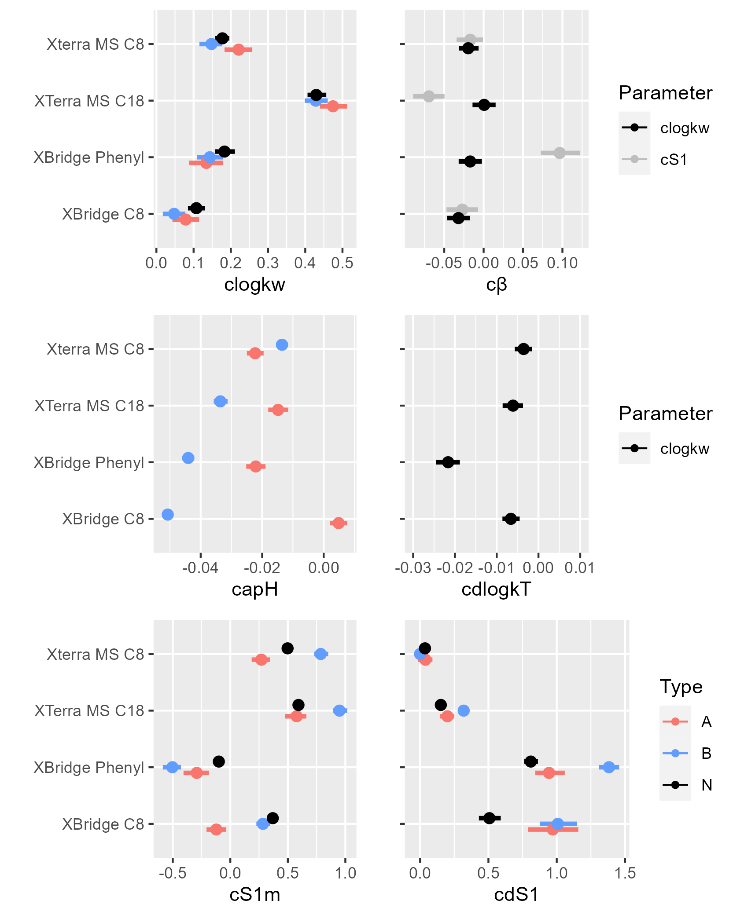


Figure 2. Summary of the marginal posterior distributions of the key population-level parameters that characterize the difference in the retention of the analytes between the indicated column and XBridge Shield RP18 column (column effects).

The log P curves for *logkw* and *S1m* exhibit slopes of 0.83 and 0.48, respectively, for XBridge Shield RP 18. The column effects on this parameter are close to zero, implying similar effects of *logP* on analyte retention. The largest effect of 0.1 is observed for the XBridge Phenyl *cS1m* parameter.

The *S1* parameter is higher in ACN than in MeOH by 0.61 for the XBridge Shield RP18 column. In addition, it is higher by 0.15, 0.81, 0.51, 0.04 for XTerra MS C18, XBridge Phenyl, XBridge C8, and Xterra MS C8, respectively. The BAV for dS1 (ωdS1) is about (0.55) and for column effects it ranges from (0.14–0.47).The *S2* parameter of the Neue equation is 0.49 in MeOH and 1.3 in ACN. The temperature effect for XBridge Shield RP18 is small and consistent with prior knowledge (−0.09 per increase in 10°C) with a BAV of 0.03. The column effects on temperature and corresponding BAV are small. The largest effect of −0.02 was observed for XBridge Phenyl. This result suggests that quantification of the temperature effect on one of the columns allows fairly precise predictions of this effect on other columns.

The *logkw* for acids and bases is lower by −0.79 and −0.97, respectively, on XBridge Shield RP18 at pH = 7 with BAV of 0.59. The column effects for that parameter are very small (from −0.04 to 0.04) with BAV ranging from 0.07–0.11. The pH effects on *logkw* for acids and bases (*apH*) are small and negative for acids (−0.03 per unit pH) and positive for bases (0.08 per unit pH) for XBridge Shield RP18. The column effects for *apH* vary across columns and range for acids from −0.02 to 0 and for bases from −0.01 to 0.05. The *apH* parameter quantifies the silanol activity.

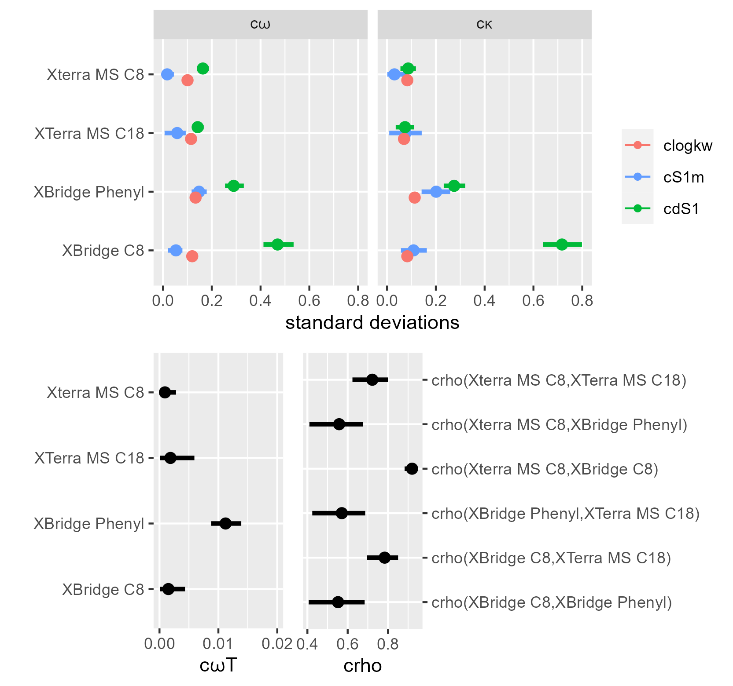


Figure 3. Summary of the marginal posterior distributions of the between analyte variability (BAV) parameters that characterize the difference in the retention of the analytes between the indicated and reference XBridge Shield RP18 columns.

The *S1m* for acids and bases is higher by 0.17 and 0.12 on XBridge Shield RP18 with a BAV of 0.69. The column effects for this parameter vary from −0.49 to 0.36, depending on the column and analyte form, with the BAV ranging from 0.04 to 0.20. The slopes for the acids and bases are different in ACN (relative to *S1m*) by 0.28 for acids and −0.67 for bases for XBridge Shield RP18, with a BAV of 0.55. The column effects for this parameter vary from −0.04 to 0.57, depending on the column and analyte form, with a BAV in the range of 0.07–0.72 (the highest value was observed for XBridge C8).

The model predictions are well calibrated with the data for all the columns, as shown in Figure S3. The individual and population predictions versus observed retention times shown in Figure S4 and S5, respectively are relatively symmetrically distributed around the line of identity. The individual prediction is very precise and close to observed data. The population predictions are also well calibrated but (as expected) are less sharp/precise. The limited-data-prediction results are shown in Figure S6. By comparing them with the population predictions, we can assess the added predictive value of the XBridge Shield RP18 data. Uncertainty chromatograms for population, individual, and limited data predictions under the same chromatographic conditions are shown in Figure S7; they illustrate the retention uncertainties for different data. Evidently, the added predictive value of XBridge Shield RP18 data is large. As an example the standard deviation of retention time predictions is reduced from ~10 min for population predictions to ~2 min for limited data predictions and further to approximately 0.1 for individual predictions. It confirms that the population predictions are very uncertain and have limited practical usefulness. The reason is that they are mostly driven by unexplained BAV *ω*, which is large. However, by adding experimental information uncertainty can be reduced. For example, the predictions based on all the experimental data are very precise. For the limited data predictions the uncertainty is almost entirely reduced for XBridge Shield RP18 column (a large amount of data collected using that particular column). For all the other columns there is still some proportion of uncertainty left; in this case, it mostly depends on c*ω*. Because *cω* is small (~0.1 for neutral forms of analyte),we can expect fairly precise predictions, i.e., we can predict the isocratic retention factor with an uncertainty of approximately 20%–25%, which might be practically useful for simple problems involving few analytes.

All the model parameters jointly affect the analyte retention. To elucidate this joint effect of the parameters, we simulated the retention factors for the typical acidic, basic, and neutral analytes with log P of 0, 3, and 6; the results are presented in Figure 4. Based on this graph, the column characteristics can be directly compared for a wide range of chromatographic conditions. We also provide various isocratic predictions (with uncertainties) for the six selected analytes to illustrate the impact of parameters on isocratic retention (Figure S8 and S9).

The model can be used in decision-making to help identify chromatographic conditions leading to the desired separation. In this study, it was illustrated using statistical decision theory. For that purpose we proposed a very simple utility function that favors shorter runs within a separation window between 2 and 40 min and ensure at least 2 min difference in the retention of the critical pair of analytes. The utility maps are presented in Figures S10 and S11, and the uncertainty chromatograms for the highest expected utility are shown in Figures 5 and 6 for individual and limited data predictions. Based on individual predictions, we can identify the chromatographic conditions that lead to large expected utilities for all the analyzed columns. The highest expected utility among the simulated chromatographic conditions was identified for Xterra Phenyl. Nevertheless, the differences in expected utilities are small suggesting that similar separations (with respect to the proposed utility) can be identified also for other columns. Based on the utility maps, we can also identify the regions of chromatographic conditions that result in similar separations.

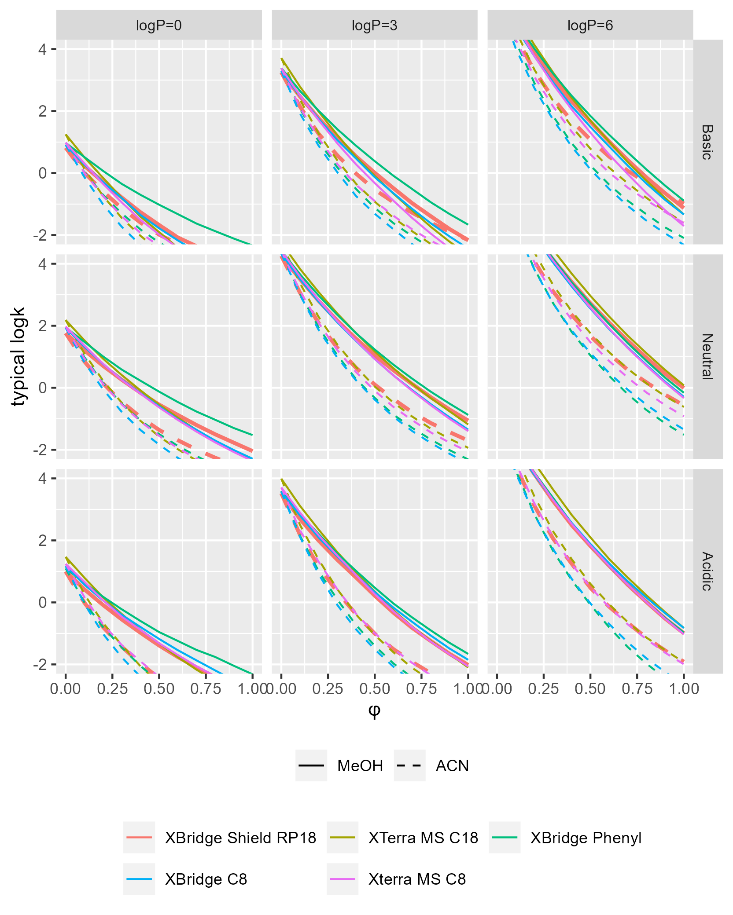


Figure 4. Graphical display of the typical retention profiles (log k vs. φ) for typical basic, neutral, and acidic forms of an analyte with log P of 0, 3, and 6 at 25oC. The uncertainty is not provided for better readability.

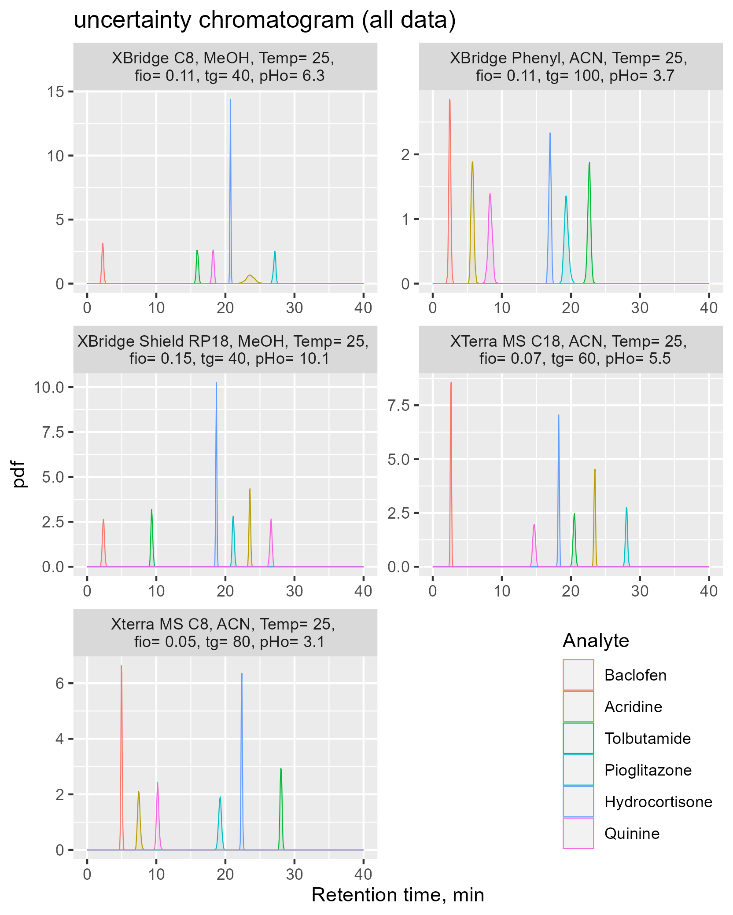


Figure 5. Optimal Bayesian-based chromatographic conditions determined from individual data prediction results.

It is also possible to identify the optimal conditions based on limited data (e.g., conditional on XBridge Shield RP18 data); that is the retention on a different column can be predicted based on our fairly good understanding of the retention of analytes for one reference column. In our scenario, the uncertainties of the resulting chromatograms are fairly small (standard deviation is in a range of few minutes for gradient conditions). However, they are not sufficient to identify the best conditions with confidence. Clearly, some additional experiments are required to improve the precision, which can be realized through various method. We can identify the best experimental design to ensure the highest informativeness of a set of additional experiments. Because the proposed model provides a priori information for a subsequent analysis, this prior information can be utilized to decrease the number of future experiments. We can also perform experiments sequentially and then refine the predicted results after every experiment.23 The uncertainty-quantifying model allows identification of chromatographic conditions for which the desired separation is plausible at any state of knowledge related to the problem; this is equivalent of having access to some preliminary data.

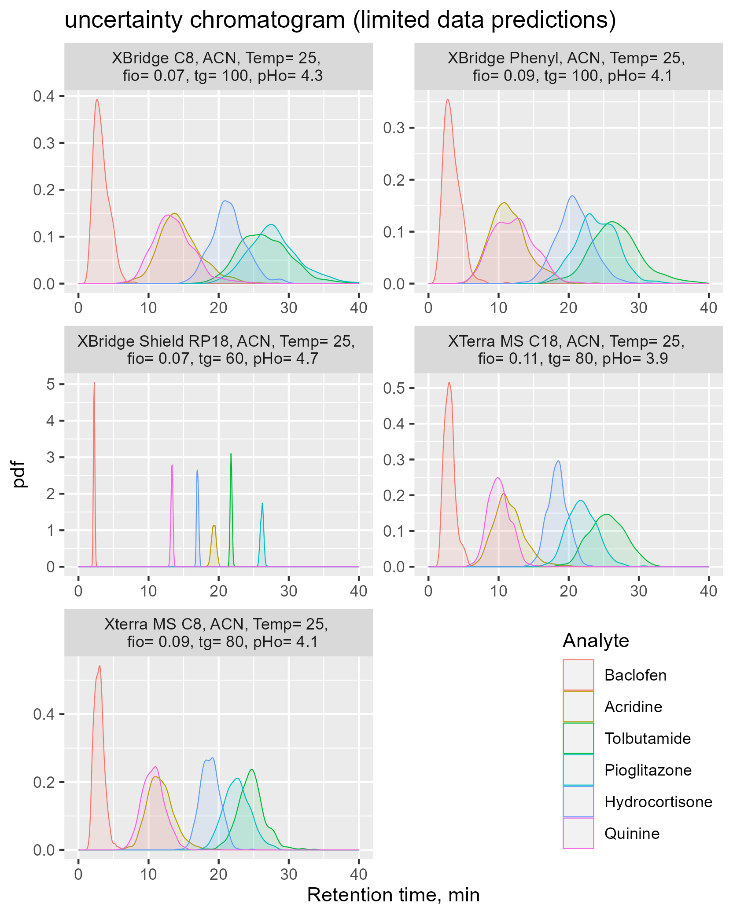


Figure 6. Optimal Bayesian-based chromatographic conditions determined from limited-data-prediction results.

The proposed model is complex, but there are still several improvements that can be considered (e.g., temperature effects for the effects of dissociation and *pKa*, BAV for *S2* and *apH*, etc.). Specifically, the S2 parameters were assumed similar across the columns; this assumption is justified because any small change in S2 can be equally well accounted for by the changes in S1. We also encountered several technical difficulties during the model building process that required simplifications to run the MCMC without any convergence issues, e.g., excluding analytes with more than two dissociations steps and analytes which were incorrectly identified (with mismatch between observed and ACD-predicted pH-profile). In the present model, the column effects were incorporated as fixed effects. It is also possible to include them as random effects and add column-level predictors. It would allow to build a model that generalizes other columns, in a similar way the current model generalize of other analytes. However, this process would require data collected for a wider range of columns. In this study, we used log P as the only predictor that linked molecular structures to chromatographic parameters. In principle, various functional groups can be added that would allow to assess the impact of molecular structure on retention in a more nuanced fashion9. Such more complex model would lead to a more detailed characterization of column properties. It is possible to extend the proposed model with the current dataset, but would be more accurate with data comprising a large number of structurally diverse analytes.

Our results confirm that precisely predicting analyte retention without a set of preliminary chromatographic measurements is challenging. Thus, any ranking of columns in terms of their usefulness in obtaining a desired separation in a situation of knowing only analyte structure is highly uncertain (one has to use population predictions). To obtain practically useful results, few measurements need to be performed using at least one stationary phase. However, even with extensive data collected for one column, the isocratic retention factor can be predicted with a precision of only ~25% in the most optimistic scenario of a neutral compound. Such precision might be sufficient for some simple problems encountered in practice, but is not sufficient for more complex problems involving several analytes of different acidic/basic characteristics. We illustrate this using a fairly difficult problem of finding the optimal separation for six analytes of different acidic/basic properties. The proposed utility function can be more complex; that is, it can be based on the cost of performing particular chromatographic experiment, or can favor the most similar/the most orthogonal separations with respect to the any reference separation. This process would enable us to answer more tailored questions.

Proposing a single number that characterizes various interactions occurring in a chromatographic system and uniquely determines the stationary-phase properties is challenging. In our opinion, it is more valuable to provide a model that quantitates the variability and uncertainty of relevant chromatographic parameters and is sufficiently complex to generalize to other chromatographic conditions, analytes, and stationary phases. Such a model can be constructed once for a particular stationary phase and then used by others to solve their specific problems. Because the proposed approach is general, it can also be used to determine all the currently used similarity metrics (if the same analytes and chromatographic conditions are considered).

# CONCLUSIONS

This pilot study demonstrates the application of a Bayesian multilevel model in the comparison of five stationary phases using large datasets of retention times collected for a wide range of gradient chromatographic conditions. This analysis method characterizes the chromatographic retention of neutral, acidic, and basic analytes and assesses the effects of pH, temperature, log P, and *pKa* organic modifier type and content on analyte retention across a range of columns. The model, along with the estimated parameters, can be used to solve various decision problems. The proposed method is a versatile tool that facilitates comprehensive assessment of stationary-phase properties and is an interesting alternative to the existing methods.

# ASSOCIATED CONTENT

## Supporting Information

The following Supporting Information is available free of charge at the ACS website:

# 1. Model description; 2. Table S1. Summary of the MCMC simulations of the marginal posterior distributions of population-level model parameters; 3. Figure S1. Raw data for six selected analytes; 4. Figure S2. Summary of the MCMC simulations of the marginal posterior distributions of the population-level model parameters; 5. Figure S3. Goodness-of-fit plots; 6. Figure S4. Individual gradient predictions; 7. Figure S5. Population gradient predictions; 8. Figure S6. Limited data gradient predictions; 9 Figure S7. Uncertainty chromatograms. 10. Figure S8. Individual isocratic predictions; 11. Figure S9. Population isocratic predictions; 12. Figure S10. Utility maps based on individual predictions; 13. Figure S11. Utility maps based on limited data predictions.

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## Author Contributions

PW and AK analyzed the data; PW wrote the paper; PW conceived the presented idea, designed the study and supervised the project.

Notes  
The authors declare no competing financial interest.

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