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Research article

Finding the best column for polar basic analytes across reversed-phase and hydrophilic interaction liquid chromatography

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ABSTRACT

Background: The chromatographic measurement of polar molecules is often surprisingly complicated. On the one hand, the standard experiments use C18 columns, which is usually unsuitable. On the other hand, these types of molecules can behave vastly differently despite their similarities. Thus, finding the right chromatographic conditions is challenging. HILIC can be an obvious choice, but C18 is still used with suitable analytes. Comparing the two methods would be advantageous, but a numerical comparison can be problematic. Thus, a simple comparing and ranking system is needed and put to use to find the best method for the separation of polar basic molecules.
Results: In this paper, nine columns with different stationary phases, from reversed-phase to hydrophilic interaction, are compared and ranked in gradient elution. The effect of pH was also considered. The measurements were repeated in acidic, near-to-neutral, and basic conditions. A straightforward system is developed to rank different stationary phases. Its foundation was peak shapes and resolutions. Every measurement condition with all the columns on the available pHs is evaluated by each property. The testing solution consisted of ten components, which aimed to cover a wide range of polar basic molecules. The comparison also focuses on these analytes. We highlighted which analyte is adaptable to different methods or which needed exclusive conditions. The resulting best column with the optimal conditions is presented and proven highly efficient for their separation.
Significance: A straightforward comparison and ranking system is developed to test multiple chromatographic columns with different stationary phases, from reversed-phase to hydrophilic interaction in gradient elution, seeking the best method for separating polar basic molecules. Nine columns were compared in multiple conditions. The best setup resulting in the competition is presented in detail, which can be applied to a wide range of analytes.

1. Introduction

Separating polar basic molecules in chromatography is usually not straightforward because reversed-phase liquid chromatography (RPLC) measurements with C18 columns are usually unsuitable due to poor retention. From a theoretical standpoint, an obvious choice

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would be the normal phase liquid chromatography (NPLC). However, the apolar eluents are considered not environmentally friendly by modern standards. Supercritical fluid chromatography (SFC) offers a solution with carbon dioxide as the eluent, but it needs specialized equipment.

Another choice is hydrophilic interaction liquid chromatography (HILIC). It is a popular technique for a good reason. The eluents from RPLC, which are already available at most labs, can be used here. Only a column change is necessary for the setup. Unfortunately, the separation of polar basic analytes can still be problematic even by this technique. The retention mechanism is highly complex. Partition, adsorption, polar interactions, hydrogen bonding, and ion exchange can all have a role in it. Furthermore, depending on the used stationary phase from bare silica, aminopropyl, diol, amide to zwitterionic the composition of the mechanism can vary significantly. It can lead to insufficient retention or unappealing peak shape (tailing) [1]. So, a comparison was made to find the best conditions. Several columns were considered from the two main techniques applied in this scenario.

For the test, a solution was prepared containing ten polar basic components. These were selected to cover a wide range of analytes regarding molecular mass and polarity. Most of them are commonly known and used compounds. Some are naturally occurring molecules like Creatinine (CRE), Agmatine (AGM), Putrescine (PUT), Spermidine (SPD), and Spermine (SPN). Others are drugs: Lamotrigine (LAM), Risperidone (RIS), and Pramipexole (PRA). The two lesser-known components are from the latter group. MI-1851 (MI) is a furin inhibitor, and it has been successfully quantified during in vitro research of a potential drug for COVID-19 [2]. N,N-dimethylaziridinium ion (DMA) is formed from DMC, which is the alkylating agent of the calcium antagonist diltiazem [3].

Only three of these analytes can be measured with relative ease. Still, complex liquid chromatography hyphenated with mass spectrometry (LC-MS) techniques with tandem mass spectrometry (MS/MS) or high-resolution mass spectrometry (HRMS) are required to do so. LAM's pharmacokinetics was studied from human plasma [4] and it was detected in effluents too [5]. Measurements of wastewater and finger prick blood included RIS[6,7]. In order to get the desired detection limits of concentrations in the wastewater example, even in-line-solid phase extraction (SPE) had to be utilized. PRA was analyzed by its own from human and mouse plasma [8,9].

The other mentioned analytes can be an even bigger challenge to measure, however, there are some scarce examples. CRE was measured in human urine with a home-made isotope dilution extractive electrospray ionization [10], and in serum on a special C18-pentafluorophenyl column [11]. For AGM, capillary chromatography with pre-column derivatization was used [12]. PUT was detected alongside SPD, and SPN from fish products by using a negatively charged ion-pairing agent, heptafluorobutyric acid [13]. The last two were also analyzed in beer with added isotope labeling and mixed-mode column of reversed phase and weak cation exchange [14].

The tendency is that usually HILIC is used [15,16], but often-times more convoluted methods are applied. For example, we see specialized columns, like Agilent Zorbax SB-Aq [17], Thermo Scientific Hypercarb porous graphitized carbon [18], or the already mentioned C18-pentafluorophenyl [11]. The case of DMA is especially unusual, because a complex method was applied, where two columns were used, switching between them during acquisition [3]. At the end of our study, an easy LC-MS method is introduced where all of the ten analytes can be measured simultaneously, selected by a thorough comparison of numerous methods and columns.

During the selection of the chromatographic columns to compare, the first one was that we deemed the best candidate based upon our previous study [19], the Agilent Zorbax RX-Sil. It is a simple, affordable bare silica column originally for NPLC. It has quite the versatility, because it has performed in the normal phase [20], HILIC[21], and also SFC[22]. Analytes measured on this column also have a diverse list. It includes the separation of purine, and pyrimidine derivatives by Kazoka [23], where conventionally packed columns like RX-Sil are compared to a monolithic column. Although, the latter can withstand higher flow rates, thus acquisition time can be shortened, RX-Sil's performance is still sufficient. Ebinger studied caffeine, sulconazole among others, and compared the long-term retention repeatability of stationary phases in SFC[24]. RX-Sil ranked 11th out of 24 tested columns. Huang separated triterpenoid saponins [22] with RX-Sil in a fast SFC method. Also, they managed a simultaneous analysis of nucleobases, nucleosides and ginsenosides [25]. Nucleobases have also been measured successfully in our laboratory on this column by Németh [19], where DNA methylation and hydroxymethylation have been the focus for potential disease biomarkers. PUT, Gamma-Aminobutyric acid (GABA), SPD, and SPN were also measured on this column in a study of astrocytic polyamine and GABA metabolism in epileptogenesis, aiming for a novel way of antiepileptic drug development [26]. Furthermore, the study of already mentioned furin inhibitor, MI was done on this column as well [2].

The main competitors for Rx-Sil were selected from two manufacturers, and two different stationary phases from both. These were from Phenomenex, the Luna HILIC, and the Kinetex F5, also from Waters, the XBridge BEH HILIC, and Amide. These are traditional HILIC phases except F5, which can be used in multiple modes. Furthermore, a fifth column was added with a unique mechanism, the Merck SeQuant ZIC-HILIC. Reversed-phase experiments were also included as a reference with two candidates. One is from the same company as the Rx-Sil, the Agilent Zorbax SB-C18, and the other is the YMC ODS-AM.

Comparing chromatographic columns is not an easy task. There are an overwhelming number of parameters to keep in mind and an abundance of methods to choose from. There is no consensus on which one is the best, and there is a lot of debate about them, even on the van't Hoff plot, which serves the very basis of most of them [27].

An additional issue is that most comparing methods use isocratic elution, but we want to test in the same conditions as the measurements, which is a gradient program. Lesellier and West summarized comparing methods in 2007, and only two of the sixteen mentioned tests used gradient elution [28]. Van Gyseghem et al. used it to determine one parameter (silanol activity) [29]. For the others, they used isocratic measurements. Only Sándi et al. used a completely gradient environment [30]. In a more recent review, where Column Selection Systems (CSS) were discussed, again only two methods utilized gradient elution out of nineteen [31]. The work of Sándi et al. is also mentioned, but it was not part of a CSS. Dolan et al. applied fundamental principles for gradient elution to find replacement (similar) columns [32], while the Kaliszan CSS also used a gradient, but only if applied to proteomics [33]. So fair to say that a gradient comparison method is a rare phenomenon.

Table 1
Summary of the 10 components.

	Name	Abbr.	Formula	Mol.weight. (g/mol)	Conc. (ng/ml)
1	Agmatine	AGM	$C_5H_{14}N_4$	130.20	100
2	Creatinine	CRE	$C_4H_7N_3O$	113.12	22.62
3	Lamotrigine	LAM	$C_9H_7N_5Cl_2$	256.09	50
4	MI1851	MI	$C_{34}H_{53}N_{15}O_6$	767.43	500
5	N,N-dimethylaziridinium ion	DMA	$C_4H_{10}N^+$	72.08	80
6	Pramipexole	PRA	$C_{10}H_{17}N_3S$	211.33	105.67
7	Putrescine	PUT	$C_4H_{12}N_2$	88.15	1000
8	Risperidone	RIS	$C_{23}H_{27}N_4O_2F$	410.49	10
9	Spermidine	SPD	$C_7H_{19}N_3$	145.25	72.63
10	Spermine	SPN	$C_{10}H_{26}N_4$	202.35	10.12

Another concern is the test analytes used as probes. It is nearly impossible to find a perfect set of suitable analytes. A testament to that is the number of feasible probes listed in the review of Zuvela et al., which is one hundred and five [31]. The ten components we used can also attest that they can behave vastly differently despite their superficial similarities. For our purpose, a method would be appropriate which is independent of probe analytes.

Even if there would be an obvious choice, these methods work in RPLC. The assortment of techniques for other stationary phases is much more limited. Our interest also includes HILIC, where comparisons are usually made for peptide and protein separation [34,35]. There is no unified test here. The comparison of Molnarove et al. is limited to resolution [35], while the work of van Dorpe et al. shows a more complex picture and still operates with fundamental parameters like plate number or peak asymmetry [34].

On top of that, our goal is to compare RPLC and HILIC columns. Every test mentioned before only works on similar stationary phases. So comparing different ones is even more rare. It can be because, most of the time, it is irrelevant. One example is a specific comparison of intra-particle diffusivity [36]. However, there are a few analytes that can function in both conditions. So, an interphase test should be advantageous.

In the end, we chose the approach of van Dorpe [34]. We selected a few fundamental parameters for the comparison. We raised the question: What are the most important features of a chromatogram? We found that the answer to that is to have adequate, gaussian-like peak shapes and sufficient separation. So, we limited the parameters to asymmetry and resolution. Columns are compared and ranked based on these two parameters.

2. Experiments and methods

2.1. Measurements

2.1.1. Standard solution

Ten standards of polar compounds are used in total. They ranged from mildly polar to ionic in polarity and from 72 to 767 g/mol in molecular weight. The details about compounds investigated are listed in Table 1. and the chemical structures are shown in Fig. 1.

We assembled a standard solution containing all of the ten components. The goal was to concoct a mixture where the chromatographic peaks had comparable heights. The components' ionization capabilities are different, so the concentrations differ. The exact values are in Table 1. To get this stock solution, three different ways had to be taken: 1. Five components were available in solid (powder) form. 1 mg/ml solutions were prepared from all five of them, then they were diluted and combined in the final solution. The dilution solvent was acetonitrile (ACN):water 50:50 + 0.1 V/V% formic acid (FA). 2. Four components (PRA, CRE, SPD, and SPN) were available in separate 50 μmol/l concentration solutions. They were diluted with the same solvent mentioned before and combined with the previous components. This leads to the noticeable difference of their concentrations in Table 1. They do not have rounded values in ng/ml (unified to all components). However, if the unit of measurement is converted into nmol/l, their concentrations become 500, 200, 0.5, and 0.5, respectively. 3. DMA was prepared on site from N,N-dimethylaminoethyl chloride hydrochloride as described by Lee et al. [3]. This procedure results in a 800 μg/ml solution. Again, it was diluted and combined in the final solution.

All of the standards were purchased from Sigma–Aldrich Chem. Co. (St. Louis, MO, USA). Except for DMA which was prepared on site as previously mentioned. ACN was obtained from VWR International (Fontenay-sous-Bois, France). Water was produced by a Milli-Q Purification System from Millipore (Burlington, MA, USA).

2.1.2. Columns

Nine columns were used in total. Seven of them were HILIC and two C18. Properties are presented in Table 2.

2.1.3. HPLC-MS/MS conditions

Chromatographic separation has been performed with the Agilent 1100 HPLC system consisting of a binary pump, column thermostat, and autosampler. Binary mobile phase were used, A: Water, B: ACN. Additives were used according to three pH levels: 1. ~2.7pH. 0.1 V/V% FA was added. The pH was not adjusted further. 2. 6.5 and 8.0 pH. 10mM ammonium formate was added and

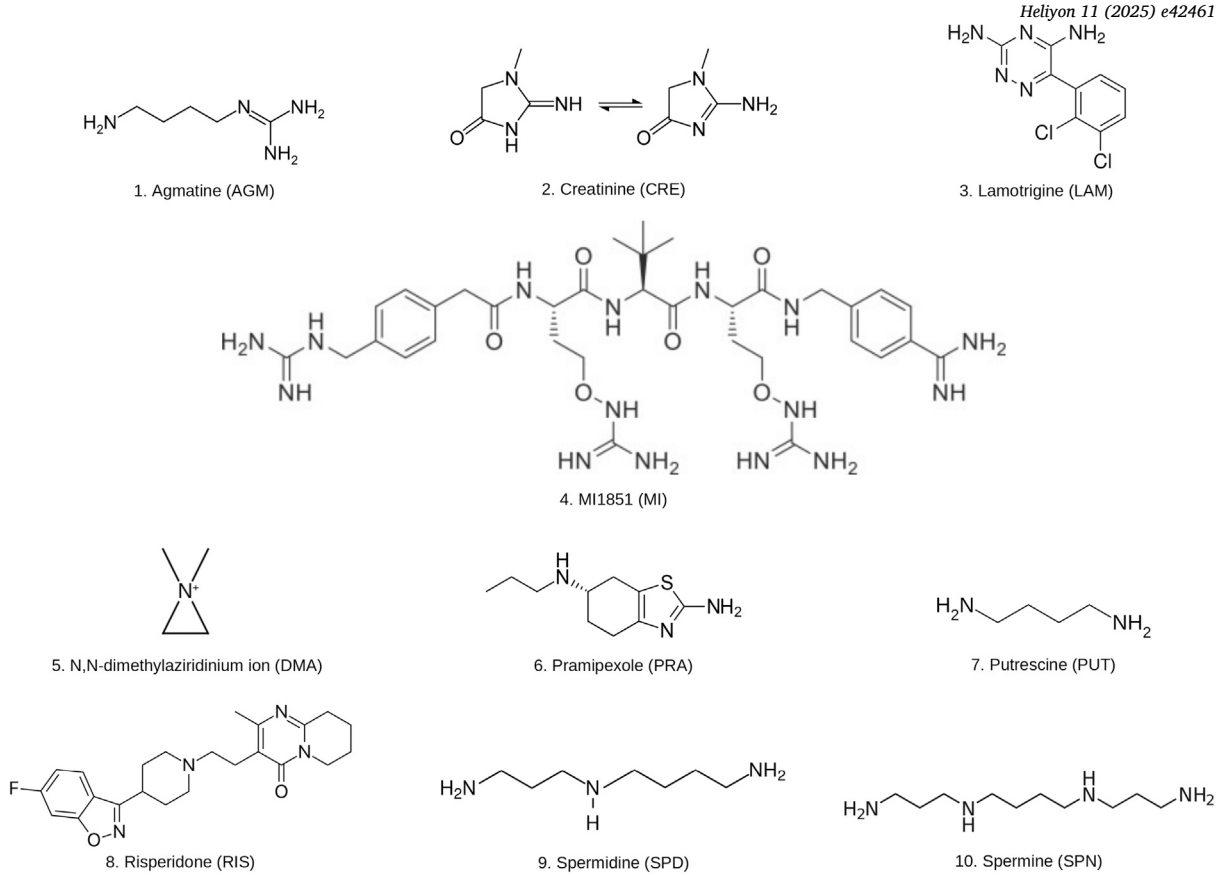

Fig. 1. Chemical structures of the 10 components.

Table 2
Properties of chromatographic columns.

	Manufacturer	Name	Abbr.	Length (mm)	Inner diameter (mm)	Particle size (μm)
1	Agilent	Zorbax RX-SIl	RXS0	250	4.6	5.0
2	Agilent	Zorbax RX-SIl	RXS1	150	2.1	5.0
3	Agilent	Zorbax SB-C18	SBC18	250	4.6	5.0
4	YMC	ODS-AM	ODS	250	4.6	5.0
5	Phenomenex	Kinetex F5	F5	100	4.6	5.0
6	Phenomenex	Luna HILIC	LunaHIL	150	3.0	5.0
7	Waters	XBridge BEH Amide	BEHA	75	3.0	2.5
8	Waters	XBridge BEH HILIC	BEHH	75	3.0	2.5
9	Merck	SeQuant ZIC-HILIC	ZHIL	150	2.1	5.0

adjusted to the correct pH. 6.5 pH was chosen according to the findings of Virgiliou et al. This pH level had the best results in their experiments of multitargeted method for metabolomics [37]. 8.0 pH was set because that is the upper limit of some columns.

In RPLC, as usual, a water rich composition was at the start. During the gradient, the ratio of apolar eluent (ACN, B) was raised. The conversion to HILIC mode was as simple as reversing the gradient, because the same eluents were used, but here the water(A) is the stronger eluent.

Gradient programs were tested and set for every column separately. Development was following the recommendations of Snyder [38] and Foster et al. [39]. Based on those works, we started out with a scouting gradient (eluent ratio from 5% to 95%), then step by step the starting-, ending ratio, flow rate and gradient steepness were optimized. Only deviations from the recommendations came from the fact of mass spectrometry (MS) detection. However, the ion source of Sciex system (detailed below) can reliably work with up to 2 ml/min, flow rate was optimized around 1 ml/min with every column. On the other hand MS helps with separation. Not every component has baseline separation, but MS adds another dimension and effectively enhances detection. It allowed us to concentrate on the optimization of peak shape, indicators are detailed below (Section: “Peak asymmetry”). The ten components reacted almost individually to any change of the gradient, so the final optimization focused on maximizing the score of our comparison method detailed below (Section: “Comparison Score”). The best performing gradient with column No. 1 is as follows: The flow rate was set

Table 3
MS MRM Parameters for quantitative analysis. It contains the selected m/z values (Q1, Q3), collision energy (CE), collision cell exit potential (CXP) and declustering potential (DP). All of the components have two transitions. The first one is the quantifier, the second is the qualifier. Dwell times were set to 45 ms for every transition.

Name	Q1(Da)	Q3(Da)	CE(V)	CXP(V)	DP(V)
AGM1	131.1	72.1	19	8	26
AGM2	131.1	114.1	11	12	41
CRE1	114.1	43.9	21	20	56
CRE2	114.1	43.1	55	20	56
LAM1	255.9	210.6	35	24	126
LAM2	255.9	144.6	49	16	126
MI1	384.7	130.8	41	14	126
MI2	384.7	346.9	21	18	126
DMA1	72.0	58.1	23	8	56
DMA2	72.0	42.0	17	16	56
PRA1	212.1	152.7	19	18	61
PRA2	212.1	110.6	30	15	100
PUT1	89.0	29.9	31	14	60
PUT2	89.0	71.5	11	12	60
RIS1	411.1	190.8	35	22	121
RIS2	411.1	109.7	65	14	121
SPD1	146.1	71.5	17	10	120
SPD2	146.1	111.5	19	12	120
SPN1	203.1	111.8	23	14	120
SPN2	203.1	128.7	17	18	120

to 1 ml/min. The linear gradient started at 30% A; it was kept for 1 min; in 4 minutes it goes up to 55% A; isocratic hold for 1 min; it goes back to 30% in 0.5 min; and it is kept for 3.5 min. Total runtime is 10 min. The other gradients are detailed in the supplementary material.

The chromatograph was coupled with the Sciex 6500 QTRAP triple quadrupole – linear ion trap mass spectrometer. The source parameters were: curtain gas, 40psi; evaporation (GS1), 50psi; drying gas (GS2), 50psi; temperature, 500 °C; ionspray voltage, 5000 V. Multiple reaction monitoring (MRM) mode was utilized. Two MRM transitions were used for every component, the details are summarized in Table 3. The more intense one was chosen for quantification (quantifier), the other transition was for qualification (qualifier). They were optimized by an automated process of the instrument, which included selecting the Q3 mass, and tuning the collision energy (CE), collision cell exit potential (CXP), and declustering potential (DP) during a continuous 7 μl/min infusion via the built-in syringe pump. Only the dwell times were adjusted afterwards. It was set to 45 ms unanimously.

2.2. Chemometrics

The parameters for column comparison were limited to only three, acceptable peaks, peak asymmetry and resolution. A combination of them was used in the final ranking.

Every calculation is carried out with self-made software. Python programming language [40] is used with the help of Spyder integrated development environment (IDE)[41].

2.2.1. Acceptable peaks

Out of ten analytes, not every one showed acceptable chromatographic peaks at every measurement. Determining the inclusion of an analyte had two steps. The first one is the peak shape. Its Difference from Gaussian (DFG), detailed in the next section, had to be lower than 1.5. The second one is retention. Even if the analyte has a nice chromatographic peak, its retention factor had to be more than 1.5. Retention factor is defined as $(t_r - T_0)/T_0$, where t_r is the retention time and T_0 is the dead time of the column. Dead times were measured by injecting ACN blank in Q1 full scan mode of the MS and determined by the solvent peak of the total ion chromatogram. Later calculations included only acceptable peaks.

2.2.2. Peak asymmetry

The most commonly used techniques for determine peak asymmetry are asymmetry factor and tailing factor. There is a third option, which is peak fitting. Usually, exponentially modified Gaussian peak (EMG) is used [42]. However, it needs far more calculating power than the previous two. The workings and connections of these methods are studied in detail by Pápai et al. [43]. The problem with the two factors is that they focus on tailing. It is more frequent, but fronting can also occur and with these calculations it is unevenly represented. So, another formula is proposed, where tailing and fronting get symmetrical values. It is expressed as $(b - a)/(a + b)$, where a and b is the two sides (front and tail) of width at 10% of height separated by the maximum point. Here, zero means the peak is identical to a Gaussian peak. A negative value represents fronting and tailing is on the positive side. With this setup, it is similar to the τ parameter of EMG. We would like to refer to it as difference from Gaussian (DFG). Fig. 2 illustrates the comparison of these

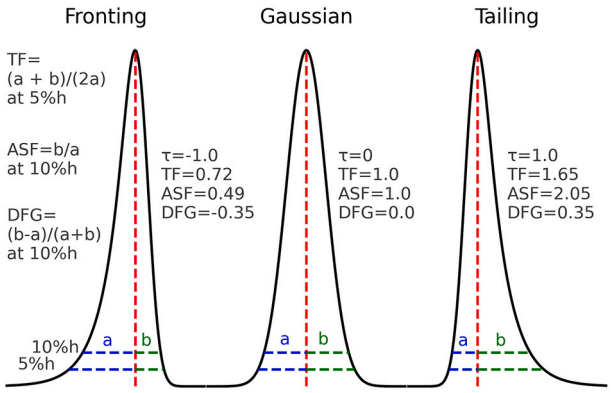

Fig. 2. Characterization of asymmetric chromatographic peaks. Commonly used functions are tailing factor (TF), which is measured at 5% of the peak height (h), and asymmetry factor (ASF), which is measured at 10%. τ is the computer-generated peaks asymmetry parameter of exponentially modified Gaussian peak (EMG). Our novel method is called difference from Gaussian (DFG), which is measured at 10%. This is the only one, that proportionately follows the original parameter.

three values on computer-generated peaks (with EMG). DFG is calculated for every peak in all of the chromatograms. Their average is considered to represent the performance of a column.

2.2.3. Resolution

The standard formula was used for the resolution, which is: $1.18 * (t_{r,2} - t_{r,1})/(FWHM_1 + FWHM_2)$, where t_r is the retention time and $FWHM$ is the full width at half maximum. It was chosen over the measurement at baseline, because the latter is perfect for Gaussian peaks, but can be problematic for asymmetric peaks. No peak pair was chosen for representation, instead, all combinations of peaks were calculated and the sum of those values was used.

2.2.4. Comparison score

Comparison between the columns can be made with either of the three parameters mentioned above separately. For ranking, though, a score was made to combine all. It is called comparison score (CS). The overall goal was to balance usability and simplicity. The number of acceptable peaks (nAP) was multiplied by the sum of resolutions (sRES) and divided by number of tested components (nTC), and the average of DFGs (aDFG), $CS = nAP/nTC * sRES/aDFG$. Two properties are put in the numerator, because they are in linear relationship with the goodness of column. aDFG is the one which is in reverse relationship, so it is in the denominator. nTC is to average nAP, and it makes this score usable between tests with different nTCs. All above results that the higher the score, the better the performance of the column in the given condition. Scaling and weighted values were considered, but they just introduced ambiguous biases and they were ultimately scrapped. CSs were calculated only from acceptable peaks (Sec. 2.2.1).

3. Results and discussion

3.1. Column comparison by individual properties

The following statements are based on the values in Table 4. It is presented in the order of final ranking, but rearranging its elements is used for the comparisons.

Nine columns were tested at three different pH levels. Determination of the best pH option for these measurements can be challenging because using different properties for comparison gives different results. Looking at nAPs (Fig. 3) shows that four out of nine columns have the maximum numbers at pH 2.7. Three columns are best at 6.5. With sRES, the balance tips over, and four columns perform better at 6.5. However, peak asymmetry (aDFG) dominates at pH 6.5 with seven out of nine columns. It is worth mentioning that aDFG and sRES depend highly on nAP. Also, performance can drop significantly between conditions. So, these results can be deceiving. For example, with RXS1, the aDFG is slightly better at pH 6.5 than 2.7 (0.34 and 0.43), but nAP drops steeply from 10 to 5. That is why we suggest preferring nAP over aDFG, a column with broader usability instead of an overall better peak shape.

Examining more closely which components can be measured on different columns shows that the bare silica stationary phase has the highest number, 10 out of 10. Interestingly, RXS1, which has the same phase as RXS0 but different dimensions, only manages 9. SPD does not have an acceptable peak here. BEHA and F5 are second with seven components. BEHH, LunaHIL, and ZHIL are in third place with a close 6. C18 stationary phases, unsurprisingly, fall back. However, nAP is not zero. Both ODS and SBC18 handle three. Even this is not obvious because these three are not the same. LAM and SPN match, but the third is PUT on ODS and RIS on SBC18. It means a column's usability is highly dependent on the given analyte, and in specific cases, C18 can also be suitable.

Peak shapes, as mentioned before at pH comparison, must be handled with caution, too. The best values are almost exclusively at pH 6.5, where nAP is lower. The winner is ZHIL with aDFG = 0.13. Indeed, PRA may have the best peak shape here out of the measurements. The other component there, CRE, also has a symmetrical peak. Contrary to PRA, it is positively wide and unfeasible for quantitative analysis. RXS0 is in second place with 0.27, calculated from 5 peaks at pH 6.5. BEHH and RXS1 are similar in this

Table 4
Ranking of chromatographic columns in different conditions. It contains the number of acceptable peaks (nAP), the average of DFGs (aDFG), the sum of resolutions (sRES), and the comparison score(CS).

	Column	pH	nAP	aDFG	sRES	CS
1	RXS0	2.7	10	0.34	376.31	1106.80
2	RXS1	2.7	9	0.43	168.9	352.10
3	BEHA	6.5	7	0.47	196.95	291.96
4	F5	2.7	7	0.47	190.46	284.99
5	BEHH	6.5	6	0.5	198.31	236.11
6	LunaHIL	8	6	0.43	115.92	163.25
7	ZHIL	8	6	0.59	20.31	20.50
8	RXS0	6.5	5	0.27	43.66	82.05
9	LunaHIL	6.5	5	0.42	53.29	62.75
10	BEHH	8	4	0.34	79.92	93.11
11	RXS1	6.5	4	0.34	30.24	35.49
12	BEHA	8	3	0.53	57.68	32.54
13	ODS	6.5	3	0.43	35.43	24.47
14	BEHA	2.7	3	0.52	40.94	23.67
15	F5	8	3	0.46	12.29	8.05
16	SBC18	2.7	3	0.39	2.37	1.84
17	SBC18	6.5	2	0.44	28.32	12.74
18	F5	6.5	2	0.41	7.45	3.67
19	ZHIL	6.5	2	0.13	1.32	1.97
20	LunaHIL	2.7	2	0.64	2.27	0.71
21	ODS	2.7	2	0.57	0.59	0.21
22	BEHH	2.7	0	-	-	-

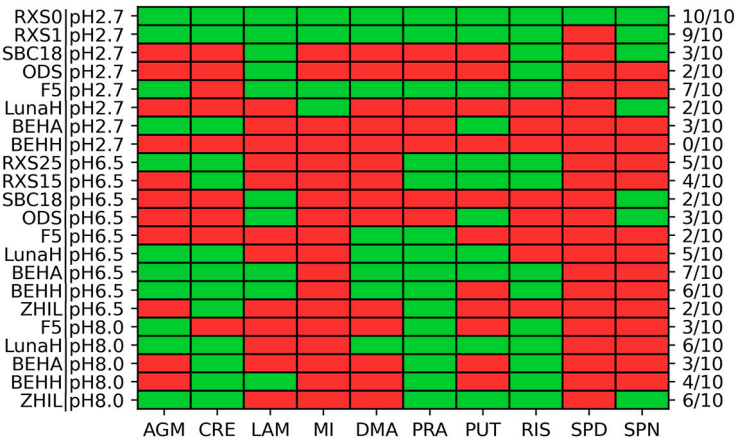

Fig. 3. Acceptable peaks (green) at every measurement. Details are in Sec. 2.2.1.

aspect. Both have an average of 0.34 from 4 peaks. RXS1 is at pH 6.5, but BEHH is at pH 8. The rest has a value between 0.39 and 0.47. BEHA places last. However, it makes its average from 7 peaks, the absolute highest from any of the columns' best aDFG values.

The sum of resolutions, again, paints another picture. RXS0 is ahead of the competition. After that, the BEHH, BEHA, F5, RXS1, and LunaHIL columns are close to each other. Maybe LunaHil lags a little between them, but other than that, their performances are comparable in this aspect, and it would be hard to pick the best out of this group. After them, the two C18 phases follow. As expected, they can not compete with HILIC columns. ZHIL is at the end of the line. A proper separation was not achievable on it. It is only suitable for PRA but not for other analytes.

3.2. Ranking of columns

After a step-by-step comparison of individual properties, an overall ranking was in order. Three values of nAP, aDFG, and sRES were combined to give a numerical basis for positioning between columns (Sec. 2.2.4). It is impossible to give an objective method. This particular metric focuses on general usability rather than best performance with a selected analyte.

However, one thing is clear. RXS0 on pH 2.7 is superior to any other option. No column comes even close to its score. If we look at any property, it is in the top two, and in most cases, it is number one. It is the single column that can produce an acceptable chromatographic circumstance for all components. It has the second-best average peak shape. Only ZHIL is better. However, considering the other facts, that is negligible. Furthermore, RXS0 has the absolute best separation.

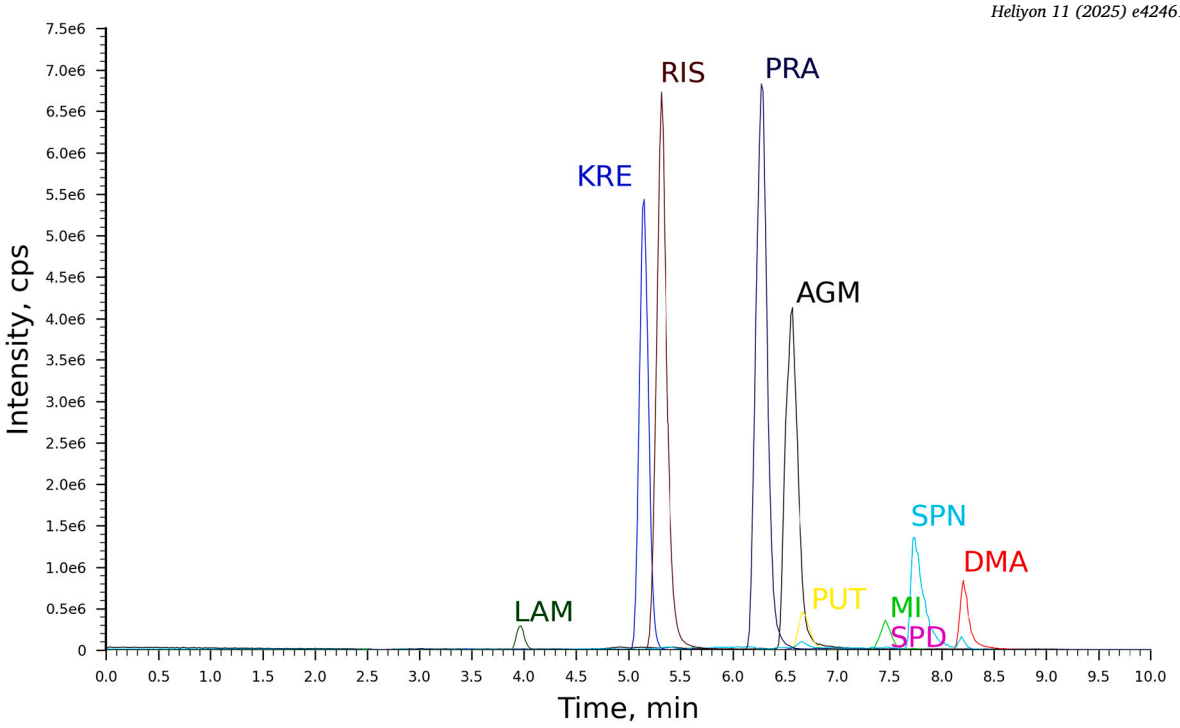

Fig. 4. Combined multiple reaction monitoring (MRM) chromatogram of the 10 components from the best performing measurement on column No. 1 (Agilent Zorbax RX-Sil 250x4.6 mm, 5um) at pH 2.7. Method parameters are in Sec. 2.1.3.

RXS1 follows closely with nine components out of ten and decent values in every aspect. After the first two places are declared, the following positions get a little blurry. Three columns have adjacent scores, not far from RXS1. They are BEHA, F5, and BEHH on pH 6.5, 2.7, and 6.5. They can manage seven or six components. So, they should be considered an adequate alternate choice. LunaHIL is next in line with a slightly lower score. Still, it is usable with six components.

Here is a gap in the list. ZHIL and the two C18, ODS, and SBC18 with the previous columns on different pHs have significantly lower values. Generally, they are considered unsuitable for measurements of polar basic analytes. However, it is worth mentioning, that in specific cases, even the reversed-phase can be an alternative to the top columns.

3.3. Best performing method

Out of the nine columns tested, RXS0 had the best results by far. A reliable method was built with it for the chromatographic separation of ten basic polar analytes. The used gradient is described in Sec. 2.1.3. The resulting chromatogram is presented in Fig. 4. A hyphenated MS technique was applied, which added another dimension of separation. It rendered the overlapping peaks measurable and allowed us to get a perfect compromise between resolution and peak shape. Although all components can only be measured with LC-MS, five components have baseline separation, and eight have less than ten percent overlap.

The advantage of the chromatographic method is its attainability. The column is simple and fairly inexpensive with the bare silica stationary phase. It is utilized in HILIC mode, which means the widely applied reversed-phase eluents, water, and ACN are suitable. The only difference is that in HILIC, water is the stronger eluent. The previous section showed that this method is best at acidic pH, which makes buffering irrelevant. Additional FA is enough to set the pH of eluents.

In the realm of basic polar analytes, the selected ten covers a wide range. So, even if this method is not suitable as the final for another particular analyte, it can serve as a starting point in the development.

4. Conclusions

Reversed-phase and HILIC columns, a total of nine, were compared on an LC-MS system with ten basic polar components. Both acidic, neutral, and basic conditions were tested. The comparison consisted of average peak shape and overall resolution. The columns were studied in both aspects and were ranked by a combined function of these properties. The RX-Sil bare silica candidate provided the definite best results. The winning method is described in detail, which ensures accessible and versatile support for similar developments.

CRediT authorship contribution statement

József Simon: Writing – original draft. **Márton Kovács:** Writing – original draft. **Pál T. Szabó:** Supervision.

Ethical approval

During the current study, no experiments were conducted on animals or humans.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pal T. Szabo reports financial support was provided by National Research, Development and Innovation Office in Hungary (TKP2021-EGA-31).

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Appendix A. Supplementary material

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42461.

Data availability

All the data used in the current study are available upon request from the corresponding author.

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