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Tracing and separating plasma components causing matrix effects in hydrophilic interaction chromatography–electrospray ionization mass spectrometry

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ABSTRACT

Matrix effects on electrospray ionization were investigated for plasma samples analysed by hydrophilic interaction chromatography (HILIC) in gradient elution mode, and HILIC columns of different chemistries were tested for separation of plasma components and model analytes. By combining mass spectral data with post-column infusion traces, the following components of protein-precipitated plasma were identified and found to have significant effect on ionization: urea, creatinine, phosphocholine, lysophosphocholine, sphingomyelin, sodium ion, chloride ion, choline and proline betaine. The observed effect on ionization was both matrix-component and analyte dependent. The separation of identified plasma components and model analytes on eight columns was compared, using pair-wise linear correlation analysis and principal component analysis (PCA). Large changes in selectivity could be obtained by change of column, while smaller changes were seen when the mobile phase buffer was changed from ammonium formate pH 3.0 to ammonium acetate pH 4.5. While results from PCA and linear correlation analysis were largely in accord, linear correlation analysis was judged to be more straight-forward in terms of conduction and interpretation.

1. Introduction

Blood plasma is a complex sample matrix with a wide variety of components which, if not eliminated or reduced by sample pretreatment, may interfere with the determination of target analytes by LC–MS. Salts, proteins, carbohydrates, amino acids, organic acids and lipids are example of components present in plasma at up to high micromolar or millimolar concentrations [1,2]. Sample components that are eluted together with the analyte may change the signal response of the analyte by altering the conditions for ionization [3–8]. Although commonly referred to as ionization suppression, the matrix effects on ionization also includes signal enhancement phenomena [9]. A matrix effect may affect assay accuracy and, if severe, detectability. Although a stable-isotope labelled internal standard (IS) in most cases will adequately compensate for the matrix effect, this may not be the case when the IS is partially separated from the analyte [10]. Potentially interfering matrix components which cannot be removed by sample pretreatment will have to be separated by chromatography or, alternatively, made less interfering by minimizing the amount of sample used for LC–MS analysis. While sample pretreatment procedures based on e.g. liquid–liquid extraction or solid phase extraction can be made selective for a narrow range of target analytes, less selective treatments such as protein precipitation have to be used in more generic methods for a wide range of analytes, which means that most of the small molecule fraction remains in the sample. Hydrophilic interaction chromatography (HILIC) has become an important alternative to reversed phase liquid chromatography (RPLC), not the least for polar compounds where RPLC offers insufficient retention [11–15]. In HILIC, a high concentration of organic modifier in water is used in combination with a hydrophilic stationary phase to retain sample components. The retention mechanisms are not fully understood but polar interactions as well as ion exchange and adsorption are thought to influence the separation [13,16]. The high content of organic solvent in the mobile phase, typically acetonitrile at 50–90%, makes HILIC well suited for electrospray ionization mass spectrometry (ESI-MS) and also makes it compatible with protein precipitation and solid phase extraction procedures. HILIC is often used in isocratic mode in order to keep separation conditions well controlled. On the other hand, gradient separation may, as in RPLC, be needed for the separation of more complex mixtures. The starting point for the present work was to apply HILIC in gradient elution mode for separation of plasma

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samples, pretreated by protein precipitation with acetonitrile. Such a method could be applied to a wide range of polar target analytes, for example unidentified polar drug metabolites, or polar endogenous compounds. Because of the dominating role of reversed phase chromatography (RPLC), most observations of matrix effects on ionization have been from such systems. Review articles on how to overcome matrix effects in bioanalytical assays using HILIC coupled to MS have been published by Mess et al. [17] and Jian et al. [18]. Much attention has been paid to phospholipids [19–21], especially lysophosphatidylcholins, which are well retained in RPLC and may interfere with more lipophilic analytes. On the other hand, matrix components with weak or no retention in RPLC, and thus easily separated from more retained analytes, have gained less attention. Such polar components are expected to exhibit more retention in HILIC systems. The aim of this work was to identify plasma matrix components which may interfere with electrospray ionization, to study their retention on different HILIC systems and to see how chromatographic selectivity can be used to master matrix effects on ionization.

2. Experimental

2.1. Chemicals

Acetonitrile (HPLC-S Grade) was obtained from, Rathburn Chemicals (Walkerburn, Scotland). Ammonium hydroxide solution (>25%), formic acid (>98%) and acetic acid (96%) were from Merck (Darmstadt, Germany). Urea and L-citrulline were obtained from Alfa Aesar (Karlsruhe, Germany). Butyryl-L-carnitine and octanoyl-L-carnitine were obtained from Larodan (Andover, MA, USA). R-(+)-propranolol hydrochloride, nicotinamide, almokalant were obtained in house. All other chemicals were from Sigma–Aldrich (Steinheim, Germany). Ultra-pure water was prepared through an in-house water purification system (Elgastat Maxima, Elga Ltd., England). Human plasma was from in house collected from healthy volunteers for exploratory purpose only.

2.2. Equipment

Columns used were: Kinetex® Silica 2.6 μm and Luna Diol 3 μm, 50 mm × 2 mm (Phenomenex), SeQuant® ZIC®-HILIC 3.5 μm, 50 mm × 2.1 mm (Merck Millipore), PC-HILIC 5 μm, 50 mm × 2.1 mm (Shiseido), XBridge Amide 3.5 μm, 50 mm × 2.1 mm (Waters), TSKgel Amide 3 μm, 50 mm × 2 mm (Tosoh Bioscience), Fortis Silica 3 μm, 50 mm × 2.1 mm (Fortis Technologies) and Diamond Hydride 4 μm, 50 mm × 2.1 mm (Cogent, MicroSolv). Column chemistries are given in Table 1. The LC–MS system used for all experiments except accurate-mass determinations consisted of an LC20-AD binary pump (Shimadzu Kyoto, Japan), a HTC PAL autosampler (CTC Analytics, Reinach, Switzerland) and an API 3000 triple quadrupole mass spectrometer with a Turbolonspray® interface (AB Sciex, Ontario, Canada) used in positive ionization mode. Data was collected and processed using Analyst® 1.4.1 software. Post-column infusion was made with a Model 22 syringe pump from Harvard Apparatus (Holliston, MA, USA) at a rate of 0.25 μL/min.

An LTQ Orbitrap mass spectrometer (ThermoFisher Scientific Bremen, Germany), was used to generate high resolution accurate-mass MS spectra using an electrospray interface. Xcalibur™ 2.0.7 software was used for data acquisition and evaluation. MS data were acquired in the positive ionization mode.

2.3. Preparation of standard solutions and plasma samples

Stock solutions (10 mmol/L) of model compounds, Table 2, were prepared and diluted in acetonitrile–water (75:25, v/v) to a final concentration of 10 μmol/L. Human K_2EDTA plasma was protein-precipitated with acetonitrile (1:4, v/v), and centrifuged.

Table 1
Columns used.

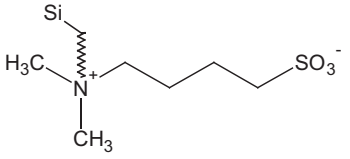
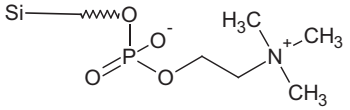
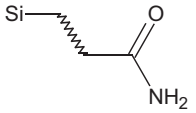
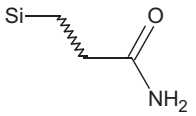
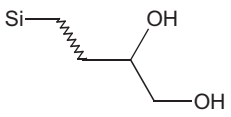
Column	Stationary phase	Chemistry
ZIC-HILIC	Zwitterionic:sulfobetaine	
PC HILIC	Zwitterionic:phosphorylcholine	
XBridge Amide	Amide ethylene-bridged silica	
TSK Gel Amide	Amide ethylene-bridged silica	
Luna Diol	Diol	
Kinetex Silica	Bare silica	Si—OH
Fortis Silica	Bare silica	Si—OH
Diamond Hydride	Hydride	Si—H

Table 2
SRM transitions used for monitoring model compounds.

	Compound	Precursor ion	Product ion
1	Almokalant[a]	353.2	261.2
2	R-(+)-propranolol[a]	260.2	116.1
3	L-Carnitine	162.0	103.0
4	Acetyl-L-carnitine	204.0	85.2
5	Butyryl-L-carnitine	232.1	85.2
6	Octanoyl-L-carnitine	288.2	85.2
7	Nicotinamide[a]	123.0	80.0
8	L-Isoleucine	132.0	69.0
9	L-Arginine	175.1	70.0
10	γ-Aminobutyric acid	104.0	87.1
11	L-Citrulline	176.2	159.4
12	Urea	61.0	44.0

[a] Only used in the evaluation of suppression/enhancement of ionization signal.

The supernatant was diluted by adding four volumes of 80% acetonitrile in water.

2.4. Chromatographic conditions

Two chromatographic buffer systems were used. Mobile phase A was a mixture of acetonitrile and 50 mM ammonium formate pH 3.0 or acetonitrile and 50 mM ammonium acetate at pH 4.5 (2:98, v/v) and mobile phase B was acetonitrile. The mobile phase systems were adjusted to pH 3.0 and 4.5 by addition of formic acid and acetic acid, respectively. Flow rate was 0.5 mL/min and the linear gradient went from 95% to 50% B between 1.0 and 7.0 min after injection. Prior to each injection, the column was conditioned at 86% acetonitrile for a few minutes, and the acetonitrile level was raised up to 95% just one minute prior to start of injection.

3. Results and discussion

Post-column infusion and selected reaction monitoring (SRM) of model compounds was used to localize regions in the chromatograms affected by the injected sample matrix. Full scan mass spectra were collected to characterize and identify interfering components. Retention data from these matrix components as well as from model compounds were collected from different HILIC systems, consisting of one of eight commercial HILIC columns and one of two mobile phases. The chromatographic selectivity of HILIC columns in gradient elution mode was evaluated by pair-wise linear correlation [16,22,23], and by principal component analysis (PCA) [11,23].

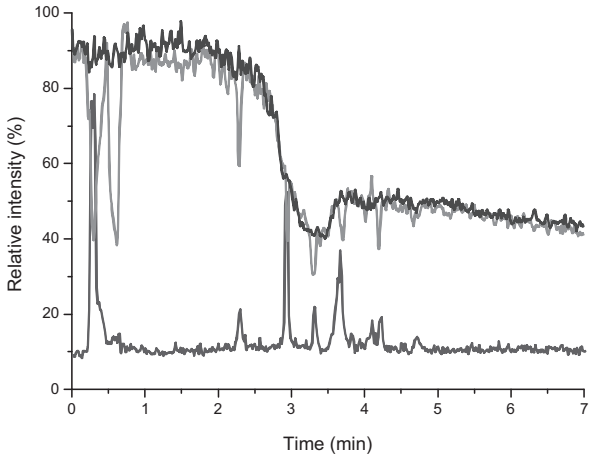


Fig. 1. Bottom: total ion current from repetitive full scans (m/z 50–950) recorded after injection of protein-precipitated plasma. Top: SRM traces (m/z 232 → 85) from butyryl-L-carnitine infused post-column, recorded after injection of protein-precipitated plasma corresponding to 0.32 μL plasma (grey) and after a blank injection (black). Column: Kinetex Silica 2.1 mm × 50 mm. Mobile phase buffer was ammonium acetate, for experimental conditions see Section 2.4.

3.1. Sample matrix components interfering with electrospray ionization

Protein precipitated plasma samples were injected and separated using gradient elution and the SRM signal from

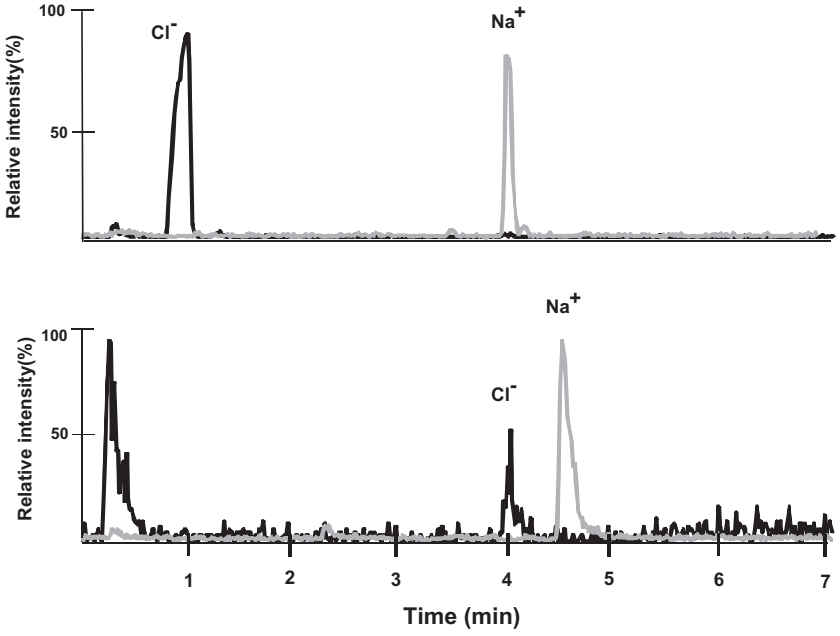
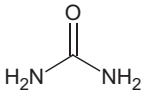
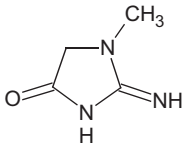
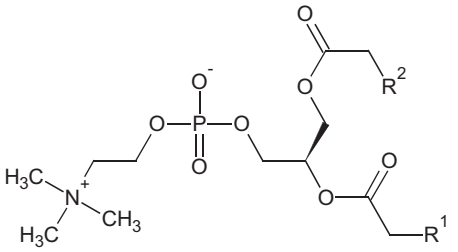
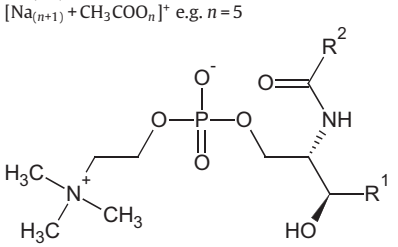
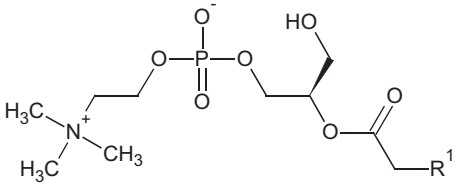
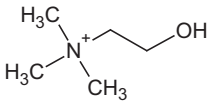
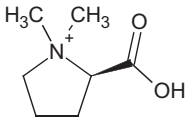


Fig. 2. Mass chromatograms, extracted from full MS scans, showing signals of chloride cluster $[(NH_4)_{14} + {}^{35}Cl_{10}\ {}^{37}Cl_3]^+$ (m/z 713) and sodium ion cluster $[Na_7 + HCOO_6]^+$ (m/z 431) from separations on Fortis Silica (upper) and ZIC-HILIC (lower) columns. A 2-μL sample, corresponding to 0.32 μL plasma, was injected. The difference in relative intensity of the chloride peak in the two chromatograms can be attributed to difference in mobile phase composition at the time of elution. Signals at 0.2–0.5 min are not from chloride ion. Mobile phase buffer system used was ammonium formate, for experimental conditions see Section 2.4.

Table 3
Components in protein precipitated plasma found to interfere with electrospray ionization.

	Major ions (MH^+)	Identity	Molecular structure/ion structure
a	713	Cluster containing Cl^-	$[(NH_4)_{(n+1)} + {}^{35/37}Cl_n]^+$ e.g. $[(NH_4)_{14} + {}^{35}Cl_{10} + {}^{37}Cl_3]^+$
b	61	Urea	
c	114	Creatinine	
d	759 783 807 835	Phosphatidylcholine (PC)	
e	431	Cluster containing Na^+	$[Na_{(n+1)} + HCOO_n]^+$ e.g. $n = 6$ $[Na_{(n+1)} + CH_3COO_n]^+$ e.g. $n = 5$
f	703	Sphingomyelin	
g	496 520 544	Lysophosphatidylcholine (Lyso-PC)	
h	104	Choline	
i	144	Proline betaine	

butyryl-L-carnitine, infused post-column, was recorded. The separation was repeated without infusion and now full-scan spectra were collected. Examples of a total-ion-current (TIC) trace and the trace of infused butyryl-L-carnitine from such a run is shown in Fig. 1. Similar traces, but not identical to those from butyryl-carnitine, were obtained from infusion of other model compounds (not shown). Spectra recorded at retention times corresponding to a decrease in the SRM signal and a coinciding positive peak in the TIC trace, were used to identify sample components. They are here discussed in the order of elution on a Kinetex Silica column (Figs. 1–3) and are listed in Table 3.

Chloride ions were detected as clusters with ammonium $[(NH_4)_{n+1} + {}^{35/37}Cl_n]^+$ with typical isotope patterns. Cluster formation is more pronounced at high acetonitrile concentrations, with clusters corresponding to $n = 13$ as major ions (m/z 807, 809, 811, 813 etc.). The identity of urea (m/z 61) was confirmed by SRM (m/z 61 → 44) and injection of reference substance. Creatinine (m/z 114) was confirmed by reference substance and high resolution MS (mass accuracy 0.3 mDa). Phosphatidylcholine (PC) (e.g. m/z 758.5, 782.5, 806.5 and 834.5 corresponding to total chain lengths and double bounds 34:2, 36:4, 38:6 and 38:3, respectively) was identified from the typical m/z values for this group of compounds. Sodium ions were detected as cluster series with formate $[Na_{(n+1)} + HCOO_n]^+$ and acetate $[Na_{(n+1)} + CH_3COO_n]^+$, with typical increments of m/z 68 and m/z 82, respectively. Sphingomyelin (m/z 703) and lyso-phosphatidylcholine (LysoPC) (e.g. m/z 496, 520 and 544 corresponding to 16:0, 18:2 and 20:5, respectively) were identified from their typical m/z values. The identity of choline

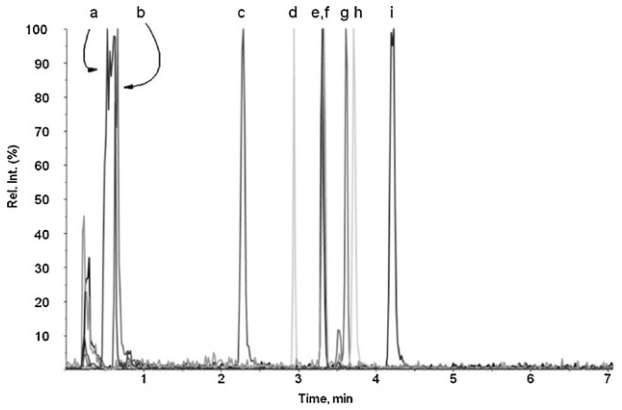


Fig. 3. Mass chromatograms for compounds in plasma disturbing the electrospray ionization, data extracted from a full MS scan. a = chloride m/z 713 (cluster ion), b = urea m/z 61, c = creatinine m/z 114, d = phosphatidylcholin, m/z 758, e = sodium ion, m/z 431 (cluster ion), f = sphingomyelin m/z 703, g = lysophosphatidylcholine, m/z 496, h = choline m/z 104, i = proline betaine, m/z 144. Column: Kinetex Silica 2.1 mm × 50 mm. Mobile phase buffer was ammonium acetate, for experimental conditions see Section 2.4.

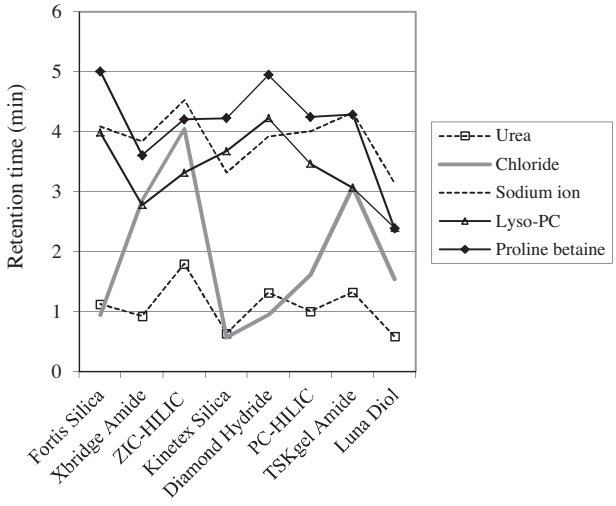


Fig. 5. Retention times (min) of five plasma components separated on eight different HILIC columns. Mobile phase buffer was ammonium formate, for experimental conditions see Section 2.4.

(m/z 104) and proline betaine (m/z 144) was confirmed by reference substance and high resolution MS (mass accuracy 0.2 mDa). Very limited separation was seen between different species of PC and LysoPC, respectively. This differs from RPLC separations where retention is substantially affected by chain lengths and number of double bonds.

A post-column infusion trace from a blank injection, overlaid in the same graph (Fig. 1), shows that ionization efficiency was not constant during the run: the increase in water content during the run resulted in decreased ionization efficiency, as is commonly observed both in HILIC and in RPLC. The decrease in signal at around 3.0–3.5 min (top black and grey traces in Fig. 1) is believed to be related to the elution of buffer components which have been accumulated earlier during the gradient cycle (unpublished results). The post-column infusion trace obtained after injection of a protein-precipitated plasma sample (corresponding to 0.3 μL plasma) shows a number of distinctive dips, corresponding to up to 80% loss of signal, which coincided with peaks in the TIC trace. The relationship between degree of analyte suppression/enhancement and the amount of plasma injected was studied by post-column infusion of the model compounds almokalant, L-carnitine, propranolol and nicotinamide during injection and separation of different dilutions of protein-precipitated plasma (almokalant, propranolol and nicotinamide were only used in the suppression/enhancement investigation). The signal response of the test compounds measured at the retention times of sodium ion and lyso-PC, respectively are shown in Fig. 4. Signal suppression or enhancement effects varied between test compounds. For example, injections of 1.25 μL protein-precipitated plasma resulted in 65% loss of signal from propranolol and more than 90% loss of signal from nicotinamide at the retention time for sodium ions. Substantial enhancement of the signal from propranolol, caused by LysoPC and also by PC and urea (not shown), was observed for smaller amounts of plasma injected (Fig. 4).

3.2. Differences in chromatographic retentivity and selectivity

Table 4 lists retention data from identified plasma components and model compound obtained from gradient separation on eight different HILIC column using ammonium formate, pH 3.0, or ammonium acetate, pH 4.5, as mobile phase buffers, in total sixteen different separations of the seventeen compounds (Tables 2 and 3). Substantial differences could be seen between columns. As an example, the retention of urea, chloride, sodium ion, lysoPC and proline betaine on the eight HILIC columns, using ammonium formate, is shown in Fig. 5. Large variations in retention were seen for chloride, which was strongly retained on ZIC-HILIC but only

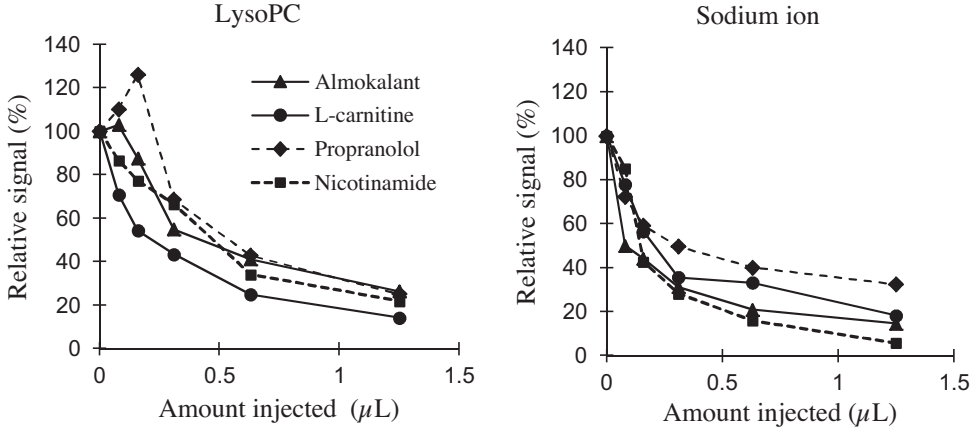


Fig. 4. Effect of Lyso-PC and sodium ions, present as matrix components in protein-precipitated plasma, on ionization of four model compounds. Amount of plasma injected refers to the corresponding volume of untreated plasma. Mobile phase buffer was ammonium acetate, for experimental conditions see Section 2.4.

Table 4
Retention data collected from eight HILIC columns using either ammonium formate pH 3.0 (prefix = F) or ammonium acetate pH 4.5 (prefix = A) and used for pair-wise linear correlation analysis and PCA. Plasma components and model compounds were eluted using a 95–50% acetonitrile gradient. For experimental conditions, see Section 2.4.

	Urea	Arginine	L-Carnitine	Iso-leucine	Citrulline	Acetyl-L-carnitine	Butyryl-L-carnitine	Octanoyl-L-carnitine	Amino-butyric acid	Chloride ion	Creatinine	PC	Sodium ion	Sphingo-myelin	Lyso-PC	Choline	Proline betaine	Mean
F-Fortis Silica	1.13	5.40	5.27	4.00	4.83	5.12	4.62	3.89	4.43	0.95	3.01	3.54	4.09	3.77	3.99	4.48	5.01	3.97
F-XBridge Amide	0.93	5.18	4.03	3.67	4.78	3.71	3.06	2.49	3.97	2.86	1.97	1.99	3.84	2.63	2.78	2.56	3.61	3.18
F-ZIC-HILIC	1.80	5.91	4.77	3.69	5.09	4.36	3.58	3.10	4.47	4.05	2.59	2.91	4.53	3.18	3.32	3.72	4.21	3.84
F-Kinetex Silica	0.64	4.86	4.75	3.42	4.20	4.50	4.08	3.54	3.86	0.57	2.29	2.93	3.32	3.33	3.68	3.72	4.23	3.41
F-Diamond Hydride	1.32	5.15	5.25	3.95	4.58	5.06	4.63	4.00	4.28	0.95	3.10	3.55	3.92	3.78	4.23	4.52	4.95	3.95
F-PC-HILIC	1.01	5.47	4.72	3.84	4.86	4.34	3.85	3.29	4.30	1.62	2.48	3.00	4.01	3.29	3.47	3.65	4.25	3.61
F-TSKgel Amide	1.33	5.58	4.69	4.00	5.10	4.32	3.61	2.92	4.37	3.08	2.76	2.55	4.32	2.89	3.07	3.64	4.29	3.68
F-Luna Diol	0.59	4.30	3.28	2.96	3.94	2.63	2.12	1.49	3.37	1.55	0.88	1.12	3.15	2.32	2.40	0.99	2.39	2.32
A-Fortis Silica	1.11	5.97	6.04	4.11	5.02	5.80	5.19	4.19	5.33	0.67	3.01	3.53	4.53	3.85	4.03	5.27	5.07	4.28
A-XBridge Amide	0.93	5.33	4.52	3.65	4.83	3.94	3.39	2.81	4.53	2.45	2.03	1.98	3.94	2.60	2.76	2.99	3.52	3.31
A-ZIC-HILIC	1.80	6.26	5.29	3.74	5.14	4.66	3.86	3.30	5.18	3.84	2.47	2.90	4.81	3.14	3.29	4.27	4.17	4.01
A-Kinetex Silica	0.63	5.74	5.51	4.00	4.58	5.04	4.52	3.68	4.63	0.41	2.28	3.00	3.78	3.31	3.49	4.52	4.25	3.73
A-Diamond Hydride	0.93	5.52	5.58	3.78	4.55	5.26	4.78	4.09	4.72	0.54	2.72	3.33	3.95	3.60	3.76	4.92	4.56	3.92
A-PC-HILIC	0.92	5.94	5.34	4.07	5.05	4.82	4.17	3.56	5.06	1.00	2.44	3.01	4.38	3.32	3.49	4.23	4.27	3.83
A-TSKgel Amide	1.31	5.98	5.23	4.12	5.21	4.66	3.94	3.20	5.03	2.43	2.76	2.56	4.49	2.90	3.08	4.22	4.28	3.85
A-Luna Diol	0.58	4.51	3.48	2.89	4.01	2.95	2.59	2.29	3.82	1.03	0.91	1.28	3.38	2.31	2.36	1.70	2.37	2.50

weakly retained on Fortis Silica and Kinetex Silica (See also Fig. 3). The weak retention of chloride ions could be explained by their repulsion from the negatively charged surface of bare silica.

Differences between chromatographic systems was also studied by pair-wise linear correlation of retention times obtained from two different separations (Tables 5 and 6). Two examples are shown in Fig. 6. From the correlation coefficient, r^2, the corresponding selectivity difference, s^2, between the two separations was calculated according to $s^2 = 1 - r^2$ [16,22]. An s^2 value of zero corresponds to no selectivity difference and a value of 1 (in practice $s^2 > 0.5$) would mean that separations are orthogonal with no correlation. The average selectivity difference was $s^2 = 0.174$ using ammonium formate, pH 3.0, and $s^2 = 0.116$ using ammonium acetate, pH 4.5, as buffers. Maximum and minimum values of the selectivity differences s^2 were 0.409 and 0.005, respectively using ammonium formate and 0.292 and 0.003 using ammonium acetate. Pair-wise linear correlation was also done comparing separations on the same column obtained with the two different buffers (Table 6). The selectivity difference ranged from $s^2 = 0.014$ for the PC HILIC and Fortis Silica columns to $s^2 = 0.039$ for the Luna Diol column, which is roughly one order of magnitude smaller than the mean selectivity difference between two columns with one and the same buffer. It should be said that the difference between the two aqueous mobile phase buffers, 50 mM ammonium formate, pH 3.0, and 50 mM ammonium acetate, pH 4.5, mixed with neat acetonitrile during gradient runs, is not very large. Changing buffer strength, type of buffer or pH further could yield larger variations in selectivity than obtained here. However, there are limitations set by hydrophilic interaction chromatography itself (a minimum ion strength required), by mass spectrometric detection (high ion strengths and non volatile buffers to be avoided) by the gradient mode (limited buffer solubility at acetonitrile levels at and above 90% acetonitrile) and by the column packing (HILIC columns have a smaller pH window than reversed-phase columns).

Column selectivity differences between columns was further evaluated by principal component analysis (PCA), using the software Simca P+ (version 12.0.1). The retention data in Table 4 were used, where columns represented compounds (variables) and rows represented chromatographic systems (observations). Thus, data from runs with ammonium formate, pH 3.0, and ammonium acetate, pH 4.5 respectively, were set up as separate observations. The retention data were centred prior to analysis by subtracting the average retention time, calculated for each observation, listed in Table 4 from each retention time. This was done to emphasize differences in chromatographic selectivity and neglecting differences between columns in general, which otherwise would dominate the outcome of the PCA. Variables were centred but not weighted. The score and loadings plots for the two first principal components, which described 78% and 10%, respectively of the total variance, are shown in Fig. 7. The scores for columns run with ammonium acetate are shifted somewhat compared to ammonium formate, but otherwise distances (differences) between columns run with the same buffer are quite similar. According to the score plot, separations on the two columns with bare silica (Fortis and Kinetex) are similar, which is not surprising, More surprising is that the retention data from the silica hydride column (Diamond Hydride) closely resemble those from the two silica columns. This is however in accord with the linear correlation data (Table 6). An explanation could be that although, on the surface of the Diamond Hydride column material, silanols have been replaced less polar hydride groups, a sufficient amount of silanols still remains, render the stationary phase properties of bare silica, such as a net negative surface charge at pH 3.0 and pH4.5. Further, according to the score plot, Luna Diol seems to be different from the other columns. The two

Table 5
Selectivity difference s^2 (upper right) and correlation coefficients, r^2 (lower left) between HILIC columns, measured with ammonium formate, pH 3.0 (a), and ammonium acetate, pH 4.5 (b). Calculated from retention data in Table 4.

a. Ammonium formate, pH 3.0

	Fortis Silica	XBridge Amide	ZIC-HILIC	Kinetex Silica	Diamond Hydride	PC-HILIC	TSKgel Amide	Luna Diol
Fortis Silica		0.287	0.349	0.006	0.005	0.048	0.215	0.338
XBridge Amide	0.713		0.034	0.285	0.344	0.115	0.021	0.050
ZIC-HILIC	0.651	0.966		0.351	0.409	0.169	0.038	0.124
Kinetex Silica	0.994	0.715	0.649		0.007	0.050	0.227	0.321
Diamond Hydride	0.995	0.656	0.591	0.993		0.076	0.268	0.390
PC-HILIC	0.952	0.885	0.831	0.950	0.924		0.080	0.158
TSKgel Amide	0.785	0.979	0.963	0.773	0.732	0.920		0.112
Luna Diol	0.662	0.950	0.876	0.679	0.610	0.842	0.888	

b. Ammonium acetate, pH 4.5

	Fortis Silica	XBridge Amide	ZIC-HILIC	Kinetex Silica	Diamond Hydride	PC-HILIC	TSKgel Amide	Luna Diol
Fortis Silica		0.201	0.267	0.009	0.003	0.033	0.126	0.213
XBridge Amide	0.799		0.041	0.164	0.224	0.090	0.023	0.041
ZIC-HILIC	0.733	0.959		0.236	0.292	0.157	0.052	0.118
Kinetex Silica	0.991	0.836	0.764		0.008	0.019	0.102	0.172
Diamond Hydride	0.997	0.776	0.708	0.992		0.042	0.147	0.232
PC-HILIC	0.967	0.910	0.843	0.981	0.958		0.049	0.094
TSKgel Amide	0.874	0.977	0.948	0.898	0.853	0.951		0.084
Luna Diol	0.787	0.959	0.882	0.828	0.768	0.906	0.916	

amide columns and the zwitterionic column ZIC-HILIC exhibit similarities while being different from the rest. The zwitterionic PC-HILIC column seems to have intermediate properties as opposed to ZIC-HILIC. It should however be noted that about 12% of the total variance is neglected in the score plot. The loadings plot (Fig. 7) show to which extent retention data from individual compounds contribute to the scores.

While pair-wise linear regression is straightforward both regarding how calculations are done and how results are interpreted, this is not quite true for PCA. Decisions have to be made whether to normalize data, in this case retention data per compound, by centering and by weighting for differences in variance, and results will to some degree depend on these decisions. Regardless of this, differences between columns in terms of average retention will largely contribute to the outcome, if not adjusted for. For the chromatographic selectivity, average retention is of secondary importance. Since the retention data in this case were from gradient separations, which means that all components will be eluted in a limited time frame, it made sense to normalize (centre) retention data for each column by subtracting average retention time from each retention time. This means that in total three decisions were taken on how to conduct PCA.

Table 6
Correlation coefficients r^2 and selectivity differences s^2 between separations on the same HILIC column using ammonium formate, pH 3.0, and ammonium acetate, pH 4.5, as mobile phase buffer. Calculated from retention data in Table 4.

Column	r^2	s^2
Fortis Silica	0.986	0.014
ZIC HILIC	0.979	0.021
Kinetex Silica	0.982	0.018
Diamond Hydride	0.984	0.016
PC HILIC	0.986	0.014
TSK Gel Amide	0.975	0.025
XBridge Amide	0.979	0.021
Luna Diol	0.961	0.039

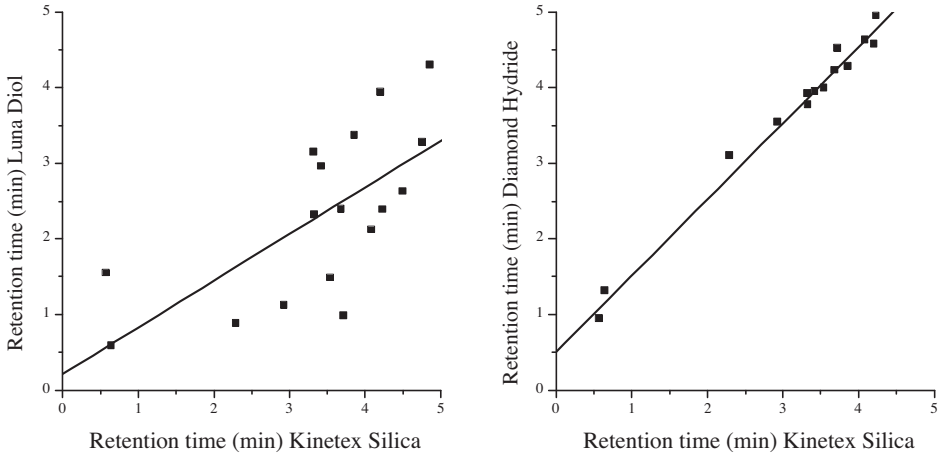


Fig. 6. Retention of 17 compounds (Tables 1 and 2) on Luna Diol (left) and Diamond Hydride (right) correlated to their retention on Kinetex Silica. The correlation coefficient r^2 was 0.678 for Luna Diol versus Kinetex Silica and 0.993 for Diamond Hydride versus Kinetex Silica column. Retention data from eight retention markers (Table 1) and nine endogenous compounds (Table 2) are used for calculations. Mobile phase buffer was ammonium formate, for experimental conditions see Section 2.4.

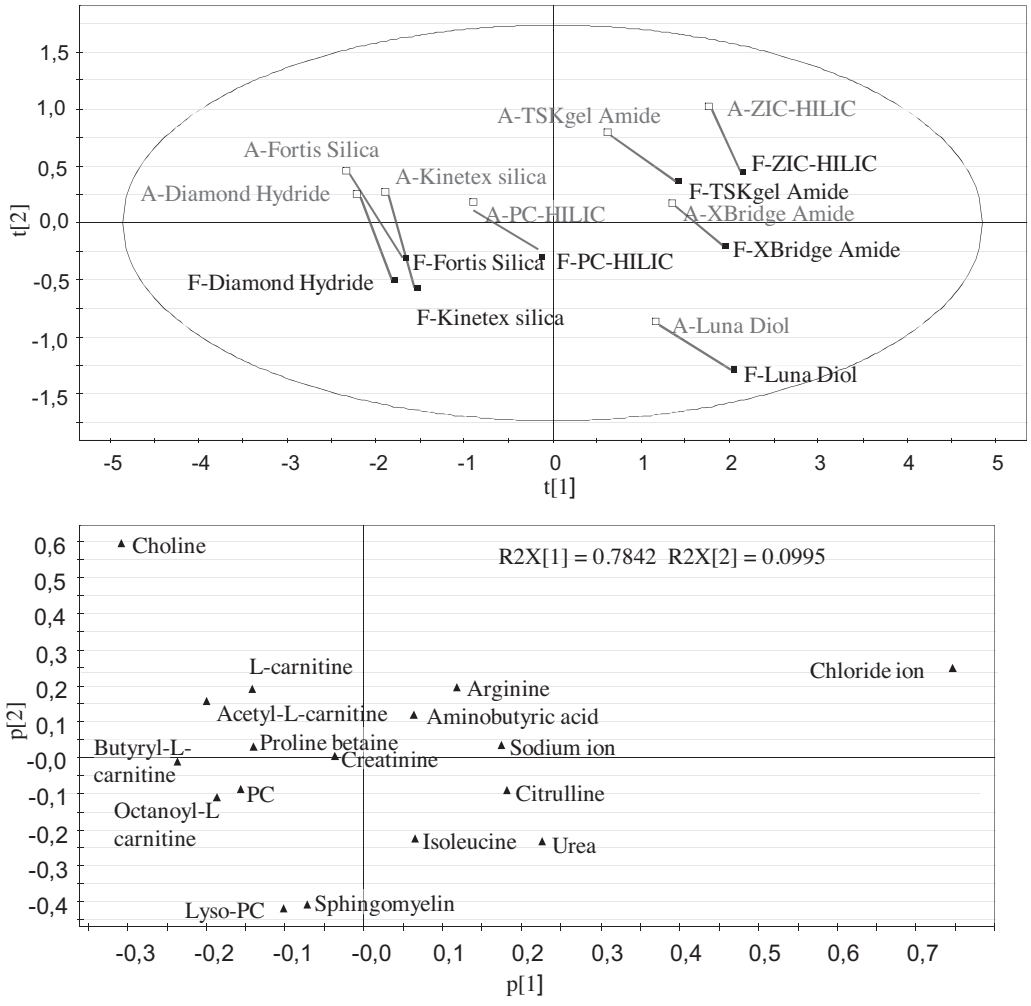


Fig. 7. Score and loadings plots of the second (t2) versus the first (t1) principal component obtained from PCA on retention data in Table 4.

4. Conclusions

Eight components of protein-precipitated plasma were identified, exhibiting significant effect on electrospray ionization of analytes in hydrophilic interaction chromatography–electrospray ionization mass spectrometry. The majority of these have in practice little influence on RPLC–ESI-MS due to low retention or, in the case of phosphocholin, excessively high retention under typical RPLC conditions. Depending on the nature of the analyte and on the amount of plasma sample injected, effects ranging from moderate enhancement or suppression to severe suppression were observed. Substantial differences in chromatographic selectivity were seen

between eight commercial HILIC columns of different chemistries, which could be exploited to alter and improve the separation of analytes from disturbing plasma components. Pair-wise linear correlation was found useful to obtain a simple quantitative measure of the difference between two separations, obtained from two different columns or from the same column under different conditions. Doing pair-wise linear correlation for all two-column combinations in a study should be a strong alternative to PCA. Matrix effects in quantitative ESI-MS may be reduced by using no more sample than needed for the instrumental analysis. If matrix components cannot be removed by clean-up procedures with reasonable effort, and if an isotope labelled internal standard is not available or does not solve the problem, then chromatography has to be tuned to avoid matrix effects. When the goal is to determine a single analyte, also small changes in selectivity may be sufficient to provide adequate separation from a disturbing component, provided that chromatographic peaks are narrow and symmetrical. Both column efficiency and selectivity should be taken into account when selecting optional columns. Buffers with different pH may also be used, as long the buffering capacity is adequate and pH is kept within the recommended range for the HILIC column.

References

[1] A.E. Burtis, D. Bruns, Clinical Chemistry and Molecular Diagnostics, 4th ed., Elsevier Saunders, St. Louis Missouri, 2006.
[2] C. Lentner, Geigy Scientific Tables, Ciba-Geigy, Basel, 1984.
[3] F. Gosetti, E. Mazzucco, D. Zampieri, M.C. Gennaro, J. Chromatogr. A 1217 (2010) 3929.
[4] D. Remane, D.K. Wissenbach, M.R. Meyer, H.H. Maurer, Rapid Commun. Mass Spectrom. 24 (2010) 859.
[5] A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. B 877 (2009) 2198.
[6] J.P. Taylor, Clin. Biochem. 38 (2005) 328.
[7] A. Cappiello, G. Famiglini, P. Palma, E. Pierini, V. Termopoli, H. Trufelli, Anal. Chem. 80 (2008) 9343.
[8] L. Tang, P. Kebarle, Anal. Chem. 65 (1993) 3654.
[9] D. Remane, M.R. Meyer, D.K. Wissenbach, H.H. Maurer, Rapid Commun. Mass Spectrom. 24 (2010) 3103.
[10] N. Lindegårdh, W. Hanpithakpong, Y. Wattanagoon, P. Singhasivanon, N.J. White, N.P.J. Day, J. Chromatogr. B 859 (2007) 74.
[11] R. Chirita, C. West, A. Finaru, C. Elfakir, J. Chromatogr. A 1217 (2010) 3091.
[12] T. Ikegami, K. Tomomatsu, H. Takubo, K. Horie, N. Tanaka, J. Chromatogr. A 1184 (2008) 474.
[13] B. Dejaegher, Y. Vander Heyden, J. Sep. Sci. 33 (2010) 698.
[14] S. Van Dorpe, V. Vergote, A. Pezeshki, C. Burvenich, K. Peremans, B. De Spiegeleer, J. Sep. Sci. 33 (2010) 728.
[15] K. Spagou, H. Tsoukali, N. Raikos, H. Gika, I.D. Wilson, G. Theodoridis, J. Sep. Sci. 33 (2010) 716.
[16] K.J. Fountain, J. Xu, D.M. Diehl, D. Morrison, J. Sep. Sci. 33 (2010) 740.
[17] C. Côté, A. Bergeron, J.N. Mess, M. Furtado, F. Garofolo, Bioanalysis 1 (2009) 1243.
[18] W. Jian, R.W. Edom, Y. Xu, N. Weng, J. Sep. Sci. 33 (2010) 681.
[19] B. Tan, A. Negahban, T. McDonald, Y. Zhang, C. Holliman, Bioanalysis 4 (2012) 2049.
[20] M. Schwalbe-Herrmann, J. Willmann, D. Leibfritz, J. Chromatogr. A 1217 (2010) 5179.
[21] J. Mess, C. Côté, A. Bergeron, M. Furtado, F. Garofolo, Bioanalysis 1 (2009) 57.
[22] U.D. Neue, J.E. O'Gara, A. Méndez, J. Chromatogr. A 1127 (2006) 161.
[23] M. Lämmerhofer, M. Richter, J. Wu, R. Nogueira, W. Bicker, W. Lindner, J. Sep. Sci. 31 (2008) 2572.