C8102 Special methods - laboratory course

# Comparison of capillary and conventional HPLC

## 1 Introduction

High performance liquid chromatography (HPLC) belongs to one of the most powerful analytical techniques. At first, sample is introduced on the cylindrical column filled with porous material (stationary phase) in the flow of solvents (mobile phase). Interaction differences in between sample and mobile and stationary phase than allow separation of individual compounds and their subsequent elution from the column.

The HPLC instrument (**Figure 1**) consists from reservoir of mobile phase, high pressure pump, sample injector, HPLC column, detector, and computer with a software allowing processing of individual peaks.

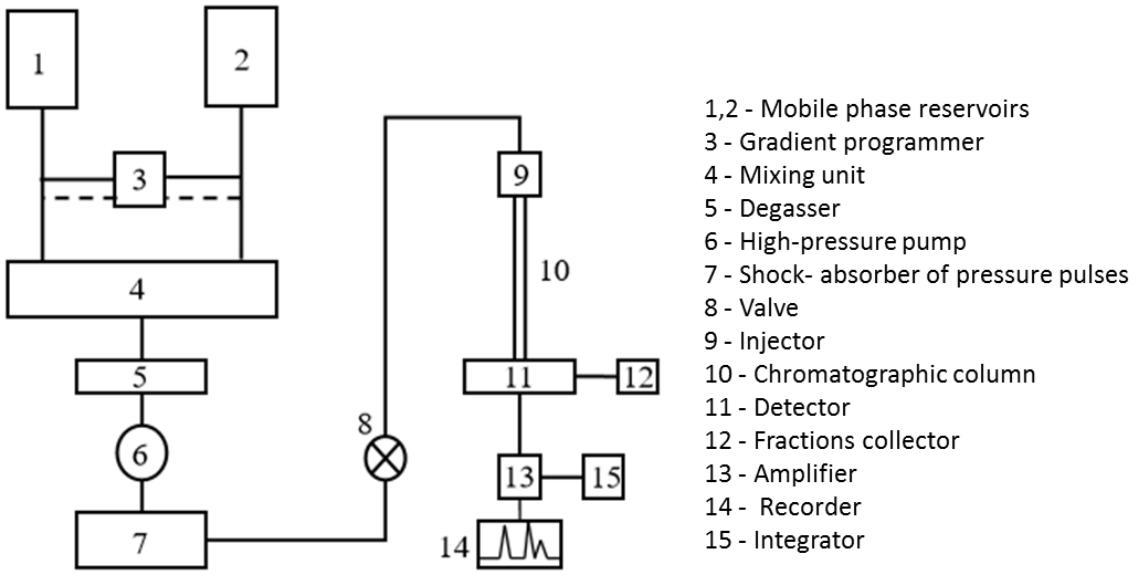



***Figure 1.*** *Basic scheme of HPLC instrumentation*

Proper selection of stationary and mobile phase is crucial in development of HPLC method and controls dominant retention mechanism. In reversed-phase mechanism the stationary phase is nonpolar (modified by C4, C8, or C18 alkyl chains) with polar mobile phase (combination of acetonitrile, methanol, tetrahydrofuran, and water). On the other hand, normal phase liquid chromatography takes place on polar

stationary phase (silicagel, aluminum oxide) with nonpolar mobile phase (hydrocarbons). The combination of both is hydrophilic interaction liquid chromatography (HILIC) with polar stationary phase and polar mobile phase. Mobile phase in HILIC is highly organic with low (2 – 20%) concentration of water. Water in the mobile phase forms aqueous layer on the surface of the stationary phase where separation takes place. Hydrophilic interaction chromatography offers retention for highly polar compounds that are not retained in reversed-phase mechanism [1].

There are two main types of stationary phases in HPLC that differ in internal structure: spherical particles and monolithic stationary phases [2]. **Figure 2A** shows main morphology difference in between these two types of columns. Monolithic stationary phases are formed by one piece of porous material that fills the whole volume of the cylindrical column. Based on the chemistry, there are two main types of the monolithic stationary phases: monolithic stationary phases based on organic polymers and inorganic monolithic stationary phases formed from sintered silica (**Figure 2B** and **2C**). While silica monoliths excel in the separation of small molecules, the main application area of (commercially available) polymer monoliths is in the fast gradient separations of large natural and synthetic polymers [3].

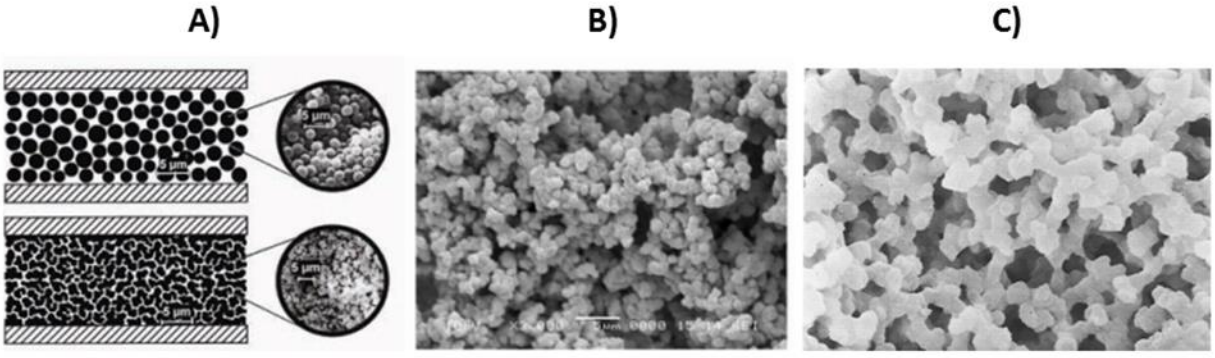



***Figure 2.*** *(A) Difference in between packed and monolithic HPLC column, (B) internal structure of polymer-based monolith, (C) internal structure of silica-based monolithic stationary phase.*

Dopamine belongs to one of the most important neurotransmitters and undesired changes in its metabolism result in serious illnesses such as depression, schizophrenia, Parkinson disease, and tumors [4]. Dominant dopamine biosynthesis pathway starts at hydroxylation of tyrosine to form 3,4-dihydroxyphenylalanine which decarboxylation leads to dopamine. Alternatively, dopamine can be biosynthesized by oxidation of tyramine. The main endproducts of dopamine degradation are 3,4-dihydroxyphenylacetic and homovanillic acids. Dopamine also serves as a precursor of epinephrine and norepinephrine. Phenolsulfotransferases and uridine diphosphoglucuronosyltransferases catalyze conjugation reactions with phosphate and glucuronic acid, respectively [5].

The aim of this laboratory task is determination of dopamine in urine by using both a conventional and capillary HPLC system. While conventional HPLC system uses commercially available column with silica-based monolithic stationary phase, in case of modular capillary HPLC system the separation takes place on home-made polymer-based monolithic capillary column.

## 2 Experimental part

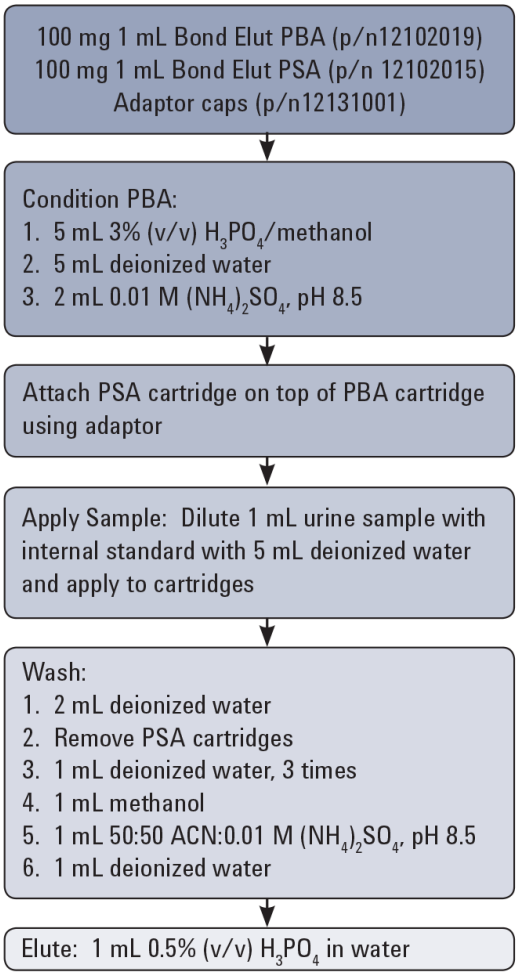
### *2.1 Chemicals*

Acetonitrile, water, trifluoracetic acid, dopamine ware purchased from Sigma Aldrich (St. Louis, MO, USA).

### *2.2 Instrumentation*

*Conventional HPLC:* Shimadzu HPLC system with high pressure pump, injector ($V_i$ = 20 μl), column compartment and detector. Column: 2 x Onyx Monolitic C18, 150 x 4.6 mm.

*Capillary HPLC:* Modular system with high pressure pump, flow splitter, injector ($V_i$ = 4 nl), and capillary detector. Column: Polymethacrylate monolithic capillary column 180 x 0.32 mm.




### *2.3 HPLC*

Teaching assistants will provide all necessary information about setup and control of the HPLC systems. In general, prepared samples are introduced in the flow of the mobile phase by using injector. After elution of the dopamine form the column, peak height and area are determined using chromatographic software. Each analysis is repeated in triplicate.

### *2.4 Solid-phase extraction*

Commercially available SPE cartridges using a pH-dependent ring formation reaction of cis-diol group with boronic acid functionality attached at the surface of SPE

material (BondElut PBA, Agilent, Palo Alto, CA, USA) are used to extract dopamine from the sample of human urine. To extract dopamine follow provided protocol.

*2.5 Determination of dopamine*

Slope of the regression line and standard deviation of residuals (difference in between fitted and experimental value) is used to determine limits of detection and quantification, as shows Eq. (1) and (2):

$$c_{LOD} = \frac{3 \cdot \sigma_r}{k} \quad (1)$$

$$c_{LOQ} = \frac{10 \cdot \sigma_r}{k} \quad (2)$$

where $\sigma_r$ corresponds to the standard deviation of residuals and *k* is a slope of the regression curve.

Calibration curve is also used to determine extracted dopamine in a human urine. To determine dopamine concentration in the urine by a standard addition method 50, 100, 150, and 200 μl of 2 mg/ml dopamine standard has been added to 1 ml of urine sample prepared as described in previous section. Determined peak areas are then plotted against concentration of dopamine (mg/l) and slope and intercept of regression line were used to determine concentration of dopamine in urine sample:

$$c_{SA} = \frac{q}{k} \quad (3)$$

Where *q* and *k* are intercept and slope of regression line, respectively.

To compare concentration of dopamine obtained by conventional and capillary HPLC systems Lord test is applied:

$$u = \frac{|X_A - X_B|}{R_A + R_B} \quad (4)$$

Where $X_A$ and $X_B$ are mean values of dopamine concentration determined on individual instruments, and $R_A$ and $R_B$ are interval of maximal – minimal values, respectively. The parameter $u$ is compared with critical tabled value for number of experiments in agreement with following table. In case $u > u_{critical}$, the difference in between two instruments is statistically significant and methods do not provide the same results.

| ***n*** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
|---|---|---|---|---|---|---|---|---|---|
| $u_{critical}$ | 1.714 | 0.636 | 0.404 | 0.306 | 0.250 | 0.213 | 0.186 | 0.167 | 0.152 |

## 3 Tasks

1) Prepare 100 ml of 5% aqueous acetonitrile with 0.1% TFA and 1000 ml of 40% acetonitrile with 0.1% TFA.
2) By subsequent dilution of provided sample of dopamine (2 mg/ml) prepare calibration curve solutions with concentration of dopamine being 0.125, 0.25, 0.5, 1.0, and 2.0 mg/ml.
3) Extract dopamine from sample of human urine by protocol provided in Experimental section.
4) Plot peak height and area vs. concentration of dopamine and construct calibration curve with linear fit of experimental data. Use error bars for each point of the curve. Use calibration curve to determine concentration of dopamine in the urine sample. Present calibration curve as an individual plot.
5) Plot peak height and area vs. concentration of added dopamine and construct curve with linear fit of experimental data. Use error bars for each point of the curve. Use standard addition method to determine concentration of dopamine in the urine sample. Present sample addition method as an individual plot.
6) Compare conventional and capillary HPLC systems in determination of dopamine. Focus on limit of detection and quantification, precision of dopamine determination, robustness and solvents consumption by both HPLC systems. Use Lord test to compare both instruments.
7) Critically evaluate obtained results.

## References


[1] Snyder, L. R., Kirkland, J. J., Dolan, J. W., *Introduction to Modern Liquid Chromatography, Third edition*, Wiley, Hoboken, New Yersey 2009.

[2] Neue, U. D., *HPLC Columns: Theory, Technology, and Practice*, Wiley, New York 1997.

[3] Urban, J., *J. Sep. Sci.* 2016, *39*, 51-68.

[4] Marc, D. T., Ailts, J. W., Campeau, D. C. A., Bull, M. J., Olson, K. L., *Neuroscience & Biobehavioral Reviews* 2011, *35*, 635-644.

[5] Meiser, J., Weindl, D., Hiller, K., *Cell Communication and Signaling* 2013, *11*, 1-18.