TEKA Archives of the Commission of Medical Sciences 2017. Vol.5, No 1.

CHROMOLITH HPLC COLUMNS IN ANALYSIS OF PLASMA AND BLOOD SAMPLES

*Michał Staniak, Ireneusz Sowa, Jan Sawicki, Janusz Polkowski, Magdalena Wójciak-Kosior **

Department of Analytical Chemistry, Medical University of Lublin, Lublin, Poland

*Corresponding author e-mail: kosiroma@wp.pl

S u m m a r y. Chromatography has a great importance in the analysis of drugs and their derivatives in samples of different origin. However, the investigation of samples such as blood or plasma is difficult because their complex matrix may clog the pores of adsorbent and spaces between sorbent particles, and it decreases column longevity. To minimalize these effects, monolithic adsorbents were introduced as an alternative to spherical stationary phases. The paper presents the application of silica based monolithic columns in the determination of drugs and their metabolites in human blood and plasma.

K e y w o r d s: high performance liquid chromatography, monolithic column, Chromolith

INTRODUCTION

High performance liquid chromatography (HPLC) is an important analytical tool to investigate biological samples of different origin. This technique is commonly applied to analyze various compounds in plant material, human tissues (blood, plasma, urine, saliva), food etc. HPLC allows to separate analyte from other components of the matrix and its high-accuracy quantification. The method offers numerous advantages such as high precision, efficiency, specificity and reproducibility. Moreover, it provides different detection options, e.g. spectrophotometric, refractometric, fluorescence, mass spectrometric, and on-line combination of a few detectors to enhance selectivity and sensitivity.

The key part of chromatographic system is a column filled with adsorbent, because proper separation process occurs during the phase inside the column. Nowadays, a lot of different stationary phases are commercially available. In adsorption chromatography the most common type of stationary phase is silica gel modified with different substituents, mostly alkyl chains.

The selection of chromatographic column depends on analytical task. The samples such as plasma and blood have a great significance for diagnostic purposes. For example, low therapeutic index of many drugs and narrow difference between therapeutic and toxic doses require accurate determination of the drug amount. The administration of a drug and analysis of blood samples afterwards allows to establish many pharmacokinetic parameters necessary to determine therapeutic dose.

However, complex matrix of these type of samples increases the susceptibility of the column to clogging. In that case, monolithic adsorbents seem to be an interesting alternative to the most common spherical stationary phases.

AIM OF THE STUDY

The subject of this study was to present the application of silica-based monolithic columns to investigate drugs and their metabolites in human blood and plasma.

Short characteristic of monolithic columns.

Monolithic columns are relatively new and less common than traditional particle-packed columns for HPLC.

Guiochon in one of his studies of monolithic columns wrote "The recent invention and development of monolithic columns is a major technological change in column technology, indeed the first original breakthrough to have occurred in this area since Tswett invented chromatography, a century ago" [1]. Since 2000 columns with monolithic fillings have been supplied by Merck and Phenomenex, the trademark Chromolith and Onyx, respectively [29]. Monolithic columns can be divided into three groups: polymer-based, silica-based, and hybrid monolithic rods [27].

Silica based monolithic columns have more benefits compared to polymer-based monoliths. They have better resistance to organic solvents and higher mechanical stability; however, their disadvantage is a narrow operating pH range (pH 2-8). In general, all monolithic columns are composed of a single piece of porous silica, often called a "silica rod" [9,18]. Macro- and mesopores are two major types of pores found in the monolithic beds (Fig.1).

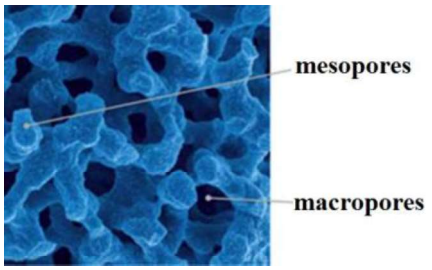

Fig.1. Structure of monolithic beds

The size of pores determines the permeability of the column [18]. The porous structure and the presence of macropores provide less resistance of the mobile phase flow, and thus lower back pressure on the column. Moreover, due to high permeability of the silica skeleton, flow rates of mobile phase may be significantly higher compared to particle-packed columns [27].

These features allow faster analysis (sometimes five or six times faster than on particle-packed column) of samples with complex matrix, especially biological ones, require less pretreatment because monolithic stationary phases are less susceptible to clogging [29].

Application of Chromolith columns in analysis of human samples

Monolithic (Chromolith) columns with 100 or 50-mm length and 4.6 mm in diameter were mostly applied for the separation of drugs and their metabolites in plasma or blood samples. In all cases, silica modified with octadecyl chains was employed as a stationary phase. Mobile phases are most often composed of water, methanol and acetonitrile at various ratios. Different modifiers such as: ammonium formate/acetate [4, 8, 6, 14, 19, 23] and organic amines (triethylamine)[2,13,15] were often added to eluents. Moreover, the mobile phases were acidified with acetic, glacial or formic acid to adjust appropriate pH. The elution was performed in gradient [17, 30, 32] or isocratic mode [3, 7, 19]. Different types of detection were used depending on the physico-chemical nature of analyte.

Frequently, the ability of compounds to fluorescence or absorbance was considered, and then, UV-VIs/diode array (DAD) or fluorescence detector was successfully used. In many cases, HPLC was coupled with mass spectrometry to enhance selectivity [14, 28, 30].

Drugs of different mechanisms of action were identified using monolithic columns, including antivirals [2, 16, 19, 28], antidepressants [6, 20, 21] and antibiotics [11, 22, 26].

Drugs from other therapeutic groups, such as antidiabetics [12], antiallergics [23], diuretics [13, 32] were also investigated; however there are only few reports in the literature.

The examples of application of monolithic Chromolith column, 100 mm- and 50 mm-length are presented in Table 1 and Table 2, respectively.

CONCLUSIONS

Monolithic filling is being used more and more often due to its beneficial properties such as the possibility of analysis at higher flow rates, and hence shorter analysis time. Moreover, higher permeability compared to particle-packed columns allows to minimalize sample pretreatment. Monolithic columns combined with modern detection methods such as mass spectrometry, provide the opportunity for rapid analysis of high selectivity and sensitivity of samples with complex matrix, e.g. blood and

plasma. Till now, different types of drugs such as antiviral, antidepressant, antibiotic, diuretic, analgesic and their metabolites have been determined and quantified with the use of monolithic columns. This type of filling is an interesting alternative to the most common spherical adsorbents.

REFERENCES

1. Al-Bokari M., Cherrak D., Guiochon G., 2002. Determination of the porosities of monolithic columns by inverse size-exclusion chromatography. Journal of Chromatography A. 975, 275–284.
2. Alebouyeh M., Amini H., 2015. Rapid determination of lamivudine in human plasma by high-performance liquid chromatography. J. Chromatogr. B. 975, 40–44.
3. Ardakani Y. H., Rouini M. R., 2007. Improved liquid chromatographic method for the simultaneous determination of tramadol and its three main metabolites in human plasma, urine and saliva. Journal of Pharmaceutical and Biomedical Analysis. 44, 1168–1173.
4. Banda J., Lakshmanan R., Prasad S., Patro Gudla S., Prudhivi R., 2015. A highly sensitive method for the quantification of fludrocortisone in human plasma using ultra-high-performance liquid chromatography tandem mass spectrometry and its pharmacokinetic application. Biomed. Chromatogr. 29, 1213-1219.
5. Bellorio K. B., Alves M. I., Filho N. A., 2013. Determination of ranitidine in human plasma by SPE and ESI-LC-MS/MS for use in bioequivalence studies. ISRN Chromatography. 2013, 7 pages.
6. Borges V., Yang E., Dunn J., Henion J., 2004. High-throughput liquid chromatography–tandem mass spectrometry determination of bupropion and its metabolites in human, mouse and rat plasma using a monolithic column. J. Chromatogr. B. 804, 277–287.
7. Brunetto Mdel R., Contreras Y., Delgado Y., Gallignani M., Estela J. M., Martin V. C., 2009. Development and validation of a rapid column-switching high-performance liquid chromatographic method for the determination of lamotrigine in human serum. J. Chromatogr. Sci. 47, 478-84.
8. Bugey A., Staub C., 2007. Application of monolithic supports to online extraction and LC-MS analysis of benzodiazepines in whole blood samples. J. Sep. Sci. 30, 2967 – 2978.
9. Cabrera K., 2004. Applications of silica-based monolithic HPLC columns. J. Sep. Sci. 27, 843 – 852.
10. Desphande N. M., Gangrade M. G., Kekare M. B., Vaidya V. V., 2010. Determination of free and liposomal Amphotericin B in human plasma by liquid chromatography–mass spectroscopy with solid phase extraction and protein precipitation techniques. J. Chromatogr. B. 878, 315–326.
11. Foroutan S. M., Zarghi A., Shafaati A., Khoddam A., Movahed H., 2007. Simultaneous determination of amoxicillin and clavulanic acid in human plasma by isocratic reversed-phase HPLC using UV detection. Journal of Pharmaceutical and Biomedical Analysis. 45, 531–534.
12. Foroutan S. M., Zargi A., Shafaati A., Khoddam A., 2006. Application of monolithic column in quantification of gliclazide in human plasma by liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 42, 513–516.
13. Galaon T., Udrescu S.,Sora I., David V., Medvedovici A., 2007. High-throughput liquid-chromatography method with fluorescence detection for reciprocal determination of furosemide or norfloxacin in human plasma. Biomed. Chromatogr. 21, 40–47.
14. Ghatol S., Vithlani V., Gurule S., Khuroo A., Monif T., Partani P., 2013. Liquid chromatography tandem mass spectrometry method for the estimation of lamotrigine in human plasma: Application to a pharmacokinetic study. Journal of Pharmaceutical Analysis. 3(2), 75–83.
15. Golabchifar Ali-A., Rouini M. R., Shafaghi B., Rezaeec S., Foroumadi A., Khoshayande M. R., 2011. Optimization of the simultaneous determination of imatinib and its major metabolite, CGP74588, in human plasma by a rapid HPLC method using D-optimal experimental design. Talanta. 85, 2320–2329.
16. Gomes N. A., Vaidyaa V. V., Pudage A., Joshi S. S., Parekh S. A., 2008. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for simultaneous determination of tenofovir and emtricitabine in human plasma and its application to a bioequivalence study. Journal of

Pharmaceutical and Biomedical Analysis. 48, 918–926.
17. Gong Z., Basir Y., Chu D., McCort-Tipton M., 2009. A rapid and robust liquid chromatography/tandem mass spectrometry method for simultaneous analysis of anti-tuberculosis drugs - ethambutol and pyrazinamide in human plasma. J. Chromatogr. B, 877, 1698–1704.
18. Guiochon G., 2007. Monolithic columns in high-performance liquid chromatography. Journal of Chromatography A. 1168, 101–168.
19. Gupta A., Guttikar S., Shah P. A., Solanki G., Shrivastav P. S., Sanyal M., 2014. Selective and rapid determination of raltegravir in human plasma by liquid chromatography–tandem mass spectrometry in the negative ionization mode. J. Pharm. Anal. http://dx.doi.org/10.1016/j.jpha.2014.10.002.
20. Hefnawy M. M., Aboul-Enein H. Y., 2004. Fast high-performance liquid chromatographic analysis of mianserin and its metabolites in human plasma using monolithic silica column and solid phase extraction. Analytica Chimica Acta. 504, 291–297.
21. Lavasani H., Giorgi M., Sheikholeslami B., Hedayati M., Reza Rouini M., 2014. A rapid and sensitive HPLC-Fluorescence method for determination of mirtazapine and its two major metabolites in human plasma. Iran J. Pharm. Res. 13(3), 853-862.
22. Louveau B., Fernandez C., Zahr N., Sauvageon-Martre H., Maslanka P., Faure P., Mourah S., Goldwirt L., 2016. Determination of rifampicin in human plasma by high performance liquid chromatography coupled with ultraviolet detection after automatized solid-liquid extraction. Biomedical Chromatography. 30, 2009-2015.
23. Muppavarapu R., Guttikar S., Rajappan M., Kamarajan K., Mullangi R., 2014. Sensitive LC-MS/MS-ESI method for simultaneous determination of montelukast and fexofenadine in human plasma: application to a bioequivalence study. Biomed. Chromatogr. 28, 1048–1056.
24. Rouini M. R., Ardakani Y. H., Hakemi L., Mokhberi M., Badri G., 2005. Simultaneous determination of clobazam and its major metabolite in human plasma by a rapid HPLC method. J. Chromatogr. B. 823, 167–171.
25. Rouini M. R., Ardakani Y. H., Moghaddam K. A., Solatani F., 2008. An improved HPLC method for rapid quantitation of diazepam and its major metabolites in human plasma. Talanta. 75, 671–676.
26. Samanidou V. F., Ioannou A. S., Papadoyannis I. N., 2004. The use of a monolithic column to improve the simultaneous determination of four cephalosporin antibiotics in pharmaceuticals and body fluids by HPLC after solid phase extraction—a comparison with a conventional reversed-phase silica-based column. J. Chromatogr. B. 809, 175-182.
27. Sharma G., Tara A., Sharma V. D., 2017. Advances in monolithic silica columns for high-performance liquid chromatography. Journal of Analytical Science and Technology. 2017, 8-16.
28. Singh O., Saxena S., Mishra S., Khuroo A., Monif T., 2011. Determination of valganciclovir and ganciclovir in human plasma by liquid chromatography tandem mass spectrometric detection. Clinical Biochemistry. 44, 907–915.
29. Sklenářová H., Chocholouš P., Koblová P., Zahálka L., Šatínský D., Matysová L., Solich P., 2013. High-resolution monolithic columns—a new tool for effective and quick separation. Analytical and Bioanalytical Chemistry. 405, 2255–2263.
30. Soni K., Patel N., Singh K., Jha A., Patel H., Gupta R., Srinivas N. R., 2016. A sensitive triple quadrupole liquid chromatography mass spectrometric method for the estimation of valproic acid in K2EDTA human plasma using furosemide as the internal standard. Drug Research. 66(12), 666-672.
31. Urbanek L., Solichova D., Melichar B., Dvořák J., Svobodova I., Solich P., 2006. Optimization and validation of a high performance liquid chromatography method for the simultaneous determination of vitamins A and E in human serum using monolithic column and diode-array detection. Analytica Chimica Acta. 573–574, 267–272.
32. Wenk M., Haegeli L., Brunner H., Krahenb S., 2006. Determination of furosemide in plasma and urine using monolithic silica rod liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 41,1367–1370.
33. Zarghi A., Foroutan S. M., Shafaati A., Khoddam A., 2006. Development an ion-pair

liquid chromatographic method for determination of sotalol in plasma using a monolithic column. Journal of Pharmaceutical and Biomedical Analysis 41, 1433–1437.
34. Zarghi A., Foroutan S. M., Shafaati A., Khoddam A., 2006. HPLC determination of omeprazole in human plasma using a monolithic column. Arzneim.-Forsch./Drug Res. 56, 382−386.
35. Zarghi A., Foroutan S. M., Shafaati A., Khoddam A., 2007. Quantification of carvedilol in human plasma by liquid chromatography using fluorescence detection: Application in pharmacokinetic studies. Journal of Pharmaceutical and Biomedical Analysis. 44, 250–253.
36. Zarghi A., Shafaati A., Foroutan S. M., Khoddam A., 2005. Development of a rapid HPLC method for determination of famotidine in human plasma using a monolithic column. Journal of Pharmaceutical and Biomedical Analysis. 39, 677–680.
37. Zarghi A., Shafaati A., Foroutan S. M., Koddam A., Madadian B., 2010. Sensitive and rapid HPLC method for determination of memantine in human plasma using OPA derivatization and fluorescence detection: application to pharmacokinetic studies. Sci. Pharm. 78, 847–856.

T a b l e 1. Examples of application Chromolith Performance RP-18e columns (100 mm x 4.6 mm) in analysis of human plasma and blood samples.

Sample/analytes	Mobile phase	Ref
Human plasma/ raltegravir, raltegravir glucuronide	10 mM ammonium formate in water, pH=3.0, and acetonitrile (30:70)	[19]
Human plasma/ amphotericin B (free and liposomal)	5 mM ammonium acetate buffer, pH adjusted to 6.0 with glacial acetic acid, acetonitrile, methanol (48/20/32) or (25/5/70)	[10]
Human plasma/ mianserin and metabolites - desmethylmianserin, 8-hydroxymianserin, mianserin-N-oxide.	25 mM dibasic sodium phosphate:acetonitrile (75:25), pH=5.3	[20]
Human plasma/ lamivudine	50 mM sodium dihydrogen phosphate and triethylamine (996:4), pH=3,2 (concentrated phosphoric acid)	[2]
Human plasma/ diazepam and metabolites: desmethyldiazepam, oxazepam,temazepam	10 mmol L^{-1} phosphate buffer (pH 2.5)–methanol–acetonitrile (63:10:27)	[25]
Human plasma/ amoxicillin, clavulanic acid	0.02 M disodium hydrogen phosphate buffer–methanol (4:96,) adjusted to pH=3.0	[11]
Human plasma/ retinol and dl-tocopherol	100% methanol	[31]
Human plasma/ carvedilol	0.01 M disodium hydrogen phosphate buffer–acetonitrile (40:60), pH=3,5	[35]
Human plasma/ gliclazide	0.01 M disodium hydrogen phosphate buffer-acetonitrile (52:48), pH=4.0	[12]
Human plasma/ famotidine	0.03 M disodium hydrogen phosphate buffer–acetonitrile (93:7), pH=6.5	[36]
Human plasma/ imatinib	methanol/acetonitrile/triethylamine/diammonium hydrogen phosphate (20:20:0.1:59.9) pH=6.25	[15]
Human plasma, saliva and urine/ tramadol and metabolites: O,N-didesmethyltramadol O-desmethyltramadol, N-desmethyltramadol	methanol:water (19:81, v/v), pH=2.5	[3]
Human plasma/ clobazam	phosphate buffer (pH=3.5; 10 mM)–acetonitrile (70:30)	[24]
Human plasma/ rifampicin	- 0.05 M acetate buffer pH 5.7 - acetonitrile	[22]
Human plasma/ mirtazapine and its two metabolites: N-desmethyl mirtazapine and 8-hydroxymirtazapine	acetonitrile:0.025 M monobasic potassium phosphate buffer adjusted to pH=3 by phosphoric acid (20:80)	[21]
Human plasma/ montelukast, fexofenadine	20 mM ammonium formate–acetonitrile, 20:80	[23]
Human plasma/ memantine	Acetonitrile, 0.025 M phosphate buffer (50:50), pH=4.6	[37]
Blood / clonazepam, N-desalkylflurazepam, diazepam, flunitrazepam, lorazepam, midazolam, nordiazepam, oxazepam	5 mM aqueous ammonium formate adjusted to pH=3 with formic acid – acetonitrile (65:35)	[8]
Human plasma/ furosemide, norfloxacin	0.015 mol/L sodium heptane-sulfonate, 0.2% triethylamine and phosphoric acid to pH=2.5–acetonitrile–methanol (70:15:15)	[13]
Human plasma/ omeprazole	0.01 mol/l disodium hydrogen phosphate buffer-acetonitrile (93:7), pH=7.1	[34]
Human plasma/ sotalol	10% acetonitrile, 0.001 M heptane sulfonic acid, 0.02 M sodium dihydrogen phosphate, water to 100%, pH= 5.5	[33]
Human plasma and urine/ furosemide	20% - 80% of acetonitrile with 0.025% acetic acid	[32]

T a b l e 2. Examples of application Chromolith Performance RP-18e column (50 mm x 4.6 mm) in analysis of human plasma and blood samples.

Sample/analytes	**Mobile phase**	**Ref**
Human plasma/ ethambutol dihydrochloride, pyrazinamide	- 0.1% trifluoroacetic acid in water - 0.1% trifluoroacetic acid in methanol	[17]
Human plasma/ valganciclovir, ganciclovir	water, trifluoroacetic acid (1 M, pH=4.4) and methanol (29.9:0.1:70)	[28]
Human plasma/ lamotrigine	acetonitrile: 5+/-0.1 mM ammonium formate solution (90:10)	[14]
Human, mouse and rat plasma/ bupropion and its metabolites hydroxybupropion, *threo*-hydrobupropion.	8 mM ammonium acetate–acetonitrile (55:45)	[6]
Human blood serum and urine/ cephalexine, cephadroxil, cefaclor, cefotaxim	sodium acetate-acetic acid buffer solution (pH=4.0) and methanol (90:10)	[26]
Human plasma/ ranitidine	aqueous 0.1% formic acid solution: methanol (50 : 50), pH=3.8	[5]
Human plasma/ valproic acid	- 0.1% v/v acetic acid in water (100%) - 0.1 % v/v acetic acid in acetonitrile (100%)	[30]
Human plasma/ fludrocortisone	2 mM ammonium formate and acetonitrile (30:70)	[4]
Human serum/ lamotrygine	20% acetonitrile in 15 mM phosphate buffer, pH=7	[7]