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- (71) Applicant (for all designated States except US):
MOROVJÁN, György [HU/HU]; Petőfi utca 164, H-1196 Budapest (HU).
- (72) Inventors; and
(71) Applicants : **MINCSOVICS, Emil** [HU/HU]; Kelenhegyi ut 15. I/1a, H-1118 Budapest (HU). **BARTOK, Tibor** [HU/HU]; Mátyás király tér 25/a, H-6725 Szeged (HU).
- (74) Common Representative: **MINCSOVICS, Emil**; Kelenhegyi ut 15. I/1a, H-1118 Budapest (HU).
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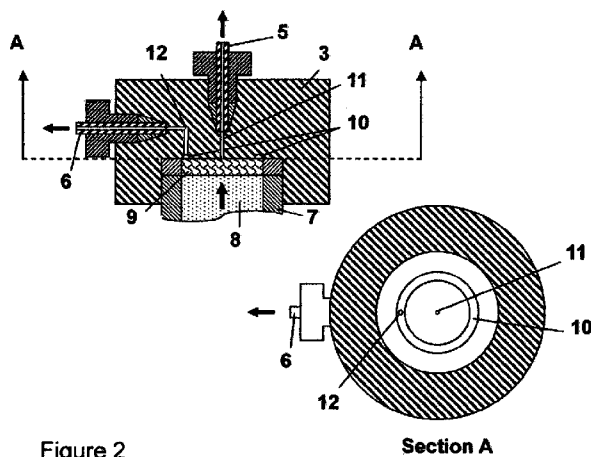


Figure 2

(57) Abstract: The invention is related to a method and apparatus for improving the performance of column chromatographic separations, which comprises introducing a sample to be separated into a mobile phase stream in a known manner and subsequently to chromatographic separation, the portion of the mobile phase exiting from the chromatographic column near the column wall is separated near the end cross-section of said chromatographic column perpendicular to the axis of said column while at the same time the portion of the mobile phase exiting from the central part of said chromatographic column near said perpendicular end cross section and a stream constituted from the centrally exiting mobile phase is subjected to analyte detection, fractionation or further separation.



Method and apparatus for improving the performance of column chromatographic separation processes

TECHNICAL FIELD OF THE INVENTION

The present invention is related to a method and corresponding apparatus for improving the performance of column chromatographic separation processes and systems used in such processes. The invention can be generally used during column chromatographic separations regardless of the scale or purpose of the separation, i.e. during analytical tests of preparative chromatographic processes.

BACKGROUND ART OF THE INVENTION

Column liquid chromatography is a separation method wherein the solute mixture to be separated dissolved in the liquid mobile phase is contacted with a fixed, non-miscible stationary phase arranged geometrically in the form of a column. During the process of separation, components of the solute mixture to be separated interact with the fixed stationary phase and according to the differences in the equilibrium constants (distribution coefficients) of the interaction, spend different time intervals associated with the stationary phase. Due to the differential distribution between the stationary and the mobile phase, components of the solute mixture to be separated leave the stationary phase separated in time. Thus, column liquid

chromatography is suitable for determining the composition of solute mixtures as well as separating and isolating the constituents of such mixtures.

Performance of a chromatographic separation method depends on several factors, including but not limited to the quality of the stationary and mobile phase, physical structure of the stationary phase. For example, in case of so-called filled column comprised of a plurality of individual particles constituting a sorbent bed, the composition, shape and size of said particles, qualitative and quantitative composition of the mixture to be separated and qualities of the sorbent bed are determining factors regarding the separation.

As mobile phase (eluent), solvents or solvent mixtures are usually applied. Stationary phases are of high-porosity, highly adsorptive solid particles or liquids immiscible with the mobile phase and coated on the surface of a solid support are generally applied.

When a filled column is used, the size of particles comprising the bed of the stationary phase is usually in the range of a few micrometers to a few hundred micrometers. Shape of the particles is usually spherical or an almost symmetrical spheroid although in some cases, particles of irregular shape are also applied. Pore size distribution, shape and character of the pores and interconnections thereof determine the adsorption processes occurring on the stationary phase as well as the kinetics of the separation significantly. Recently stationary phases have become available which comprise a porous adsorptive layer („shell”) produced on the surface of an essentially non-porous particle („core”). Chemically stationary phases are

usually particles of chemically modified inorganic or organic polymers, e.g. silica (hydrated silicic acid), alumina (aluminium oxide), zirconia, styrene-divinylbenzene copolymers, or chemically modified derivatives thereof (e.g. alkyl-modified silica). There are stationary phases disclosed in the state of the art which comprise one single porous organic or inorganic block-polymerized adsorbent block (a monolithic phase) rather than individual particles.

Analytical as well as preparative column chromatographic separations including process chromatography are characterized by the sample amount (weight) separated on the column (sample capacity), separation speed (number of samples separated in a time unit) and resolution characteristic to the separation of subsequently eluted analytes. According to the relationships known in the state of the art, resolution is a function of the distribution coefficients of the target component and the component having its distribution coefficient closest to that of the target component as well as the band (elution zone) broadening occurring in the separation process. Band broadening during the elution from a chromatographic column can be characterized by efficiency (number of theoretical plates, height equivalent to a theoretical plate, HETP). Thus, performance of a chromatographic system or a chromatographic column can be characterized by band broadening and comparison is possible by using a set of test analytes or by having test conditions fixed, between analytes as well.

It is known in the state of the art that the distribution coefficient (or retention factor) between the stationary and mobile phase and the selectivity between the critical pair of the compounds (i. e. the ratio of the distribution

coefficients of the target compound and the compound having the most closely related distribution coefficient) can be influenced by varying the quality of the stationary phase, the composition of the mobile phase or the temperature. Under given environmental conditions (temperature, pressure), the retention factor is determined by the quality of the stationary phase and the composition of the eluent for a given compound.

It is also known for a long time that band broadening resulting from diffusion and convective phenomena during a column chromatographic separation influences sample capacity, separation speed and resolution as well. Thus, during the development of chromatographic separation methods and the corresponding apparatus, reducing band broadening is desirable.

It has been observed that when an adsorbent bed comprising individual particles filled into a chromatographic tube thus comprising a chromatographic column is used, separation efficiency of the column is very significantly influenced by the spatial arrangement of the particles in the sorbent bed. Due to this experience, parties involved in the manufacturing of chromatographic columns endeavored producing the most ordered, regularly arranged sorbent bed during the column filling operations.

It is known for a long time that the axial inhomogeneity (inhomogeneity in the direction of the symmetry axis of the column; this direction is identical to the direction of the eluent flow) of the sorbent bed in the chromatographic column has significant effect for the separation performance. In order to reduce this effect, column filling methods suitable for producing high packing density sorbent bed and axial compression technology were

introduced. However, during the production of state-of-the-art high efficiency columns, optimized column filling methods play an important role.

Knox and coworkers (J.H. Knox, G.R. Laird and P.A. Raven, *Interaction of radial and axial dispersion in liquid chromatography in relation to the "infinite diameter effect"*. J. Chromatogr., **122** (1976) 129-145), and Eon (C.H. Eon, *Comparison of broadening patterns in regular and radially compressed large-diameter columns*. J. Chromatogr., **149** (1978) 29-42.) provided proof for the first time for the phenomenon that the chromatographic sorbent bed can not be regarded as homogeneous in radial direction (perpendicular to the symmetry axis of the column as well as the direction of the mobile phase flow) either. They demonstrated that the residence time and efficiency in the same perpendicular plane to the column symmetry axis at different distances from said axis is significantly different. Namely, the residence time is shorter, efficiency is lower in the partial volume of the sorbent bed situated close to the column wall than in a sorbent volume residing at a shorter distance to the column longitudinal symmetry axis.

Farkas and coworkers (T. Farkas, M.J. Sepaniak, G. Guiochon, *Column radial homogeneity in high-performance liquid chromatography*. J. Chromatogr. A, **740** (1996) 169-181.) showed also that by determining the efficiency for bands eluting from the central volume of the sorbent bed and from regions close to the column wall, significantly lower efficiency can be obtained for the band sections eluting from the sorbent bed situated in the proximity of the column wall than for band sections eluting from the central

section of the sorbent bed. They found that the efficiency of the sorbent bed situated close to the longitudinal symmetry axis of the column can exhibit even 100%- higher efficiency than the sorbent bed located in the proximity of the column wall. This phenomenon is attributed to the inhomogeneity of the sorbent bed in the vicinity of the wall of the chromatographic column.

Guiochon and coworkers (G. Guiochon, *The limits of the separation power of unidimensional column liquid chromatography*. J. Chromatogr. A, **1126** (2006) 6–49; R.A. Shalliker, B.S. Broyles, G. Guiochon, *Physical evidence of two wall effects in liquid chromatography*. J. Chromatogr. A, **888** (2000) 1–12.) disclosed that neither the flow rate of the eluent, nor the efficiency is constant in radial direction in a filled chromatographic column. Due to the inhomogeneity of the sorbent bed, the flow rate close to the column wall is lower than in the central section of the sorbent bed (the opposite trend was observed by Knox and coworkers due to wet and dry column filling methods), HETP having inverse relationship to efficiency is higher (HETP, Height Equivalent to a Theoretical Plate) (in accordance to the observation of Knox and coworkers). Similar phenomena were observed in the case of columns containing monolithic silica stationary phase (K.S. Mriziq, J.A. Abia, Y. Lee, G. Guiochon, *Structural radial heterogeneity of a silica-based wide-bore monolithic column*. J. Chromatogr. A, **1193** (2008) 97–103).

Filled preparative chromatographic columns exhibit similar effects. It has been found that the uneven flow rate distribution resulting from radial inhomogeneity of the sorbent bed contributed significantly to the dispersion (i. e. broadening) of the eluting bands (U. Tallarek, E. Baumeister, K. Albert, E. Bayer, G. Guiochon, *NMR imaging of the chromatographic process*

migration and separation of bands of gadolinium chelates, J. Chromatogr. A **696** (1995) 1-18.; U. Tallarek, K. Albert, E. Bayer, G. Guiochon, *Measurement of transverse and axial apparent dispersion coefficients in packed beds*. AIChE J. **42** (1996) 3041-3054.).

Recently Tallarek and coworkers studied chromatographic columns of 0.1 mm diameter filled with 2.6 μm particle diameter spherical core-shell adsorbent and have found that the flow resistance (pressure drop) measured in a region close to the column wall was higher than that measured for the central section of the sorbent bed, resulting from differences of extrinsic porosity. (S. Bruns és U. Tallarek, *Physical reconstruction of packed beds and their morphological analysis: Core-shell packings as an example*. J. Chromatogr. A, **1218** (2011) 1849–1860.)

It is apparent from the studies discussed above that there is a significant need for chromatographic columns having stabile and homogeneous sorbent bed while at the same time exhibiting low band dispersion. Although stabile high packing-density chromatographic columns having good separating efficacy are available by applying up-to-date column filling methods and axial or radial compression technology, methods available in the state of the art for reducing the effect of radial inhomogeneity on separation performance show several disadvantages.

US Patent No. 3966609 discloses a column chromatographic apparatus comprising a piston at least one end of the chromatographic column rather than a fixed column end piece (outlet element), said piston being moveable in the direction of the longitudinal axis (i.e. axially) of the chromatographic

column in order to decrease band broadening originating from the volume change of the stationary phase. Ports or connectors are provided for mobile phase and sample introduction to and withdrawal from the cross-section of a piston. The disadvantage of the method resides in the fact that no moveable piston can be used at the relatively high pressure used during high-performance column chromatographic separations. Furthermore, such an arrangement neither prevents the loss of efficiency caused by the poorly arranged sorbent bed near to the column wall nor that caused by radial sorbent inhomogeneity. (G. Guiochon, T. Farkas, H. Guan-Sajonz, J.-H. Koh, M. Sarker, B.J. Stanley, T. Yun, *Consolidation of particle beds and packing of chromatographic Columns*. J. Chromatogr. A **762** (1997) 83-88).

US Patent No. 4250035 discloses a method and apparatus for compensating for dead volume occurring near the column wall as well as for the inhomogeneous arrangement of the sorbent bed at the column wall to a certain degree. According to the method, the tubular part of the chromatographic column comprising the column wall is manufactured from a deformable material and a radial pressure is exerted from outside to said cylindrical column wall. During the use of such radial compression columns, the radial pressure has to be kept strictly constant, since changes of the pressure can significantly alter the performance of the chromatographic system.

According to published Japanese Patent Application No. 56044376, an auxiliary flow of the mobile phase is applied for reducing the loss of efficiency due to dead volume and inhomogeneity of sorbent bed occurring in the proximity of the column wall. Said auxiliary mobile phase flow is

introduced into the chromatographic column near the column wall at the sample introduction side independently from the means of sample introduction in order to prevent solute diffusion to the vicinity of the inhomogeneous sorbent bed volume close to the column wall, thus approximating the so-called „infinite diameter column” effect. Mobile phase containing analytes eluting from the central cross section of the column close to the longitudinal axis is directed to the detector. The flow eluting from the inhomogeneous bed free from analytes close to the column wall is separated and discarded or recycled, thus said region of the column neither participate in the separation nor directed to the detector. An essentially similar apparatus and method has been disclosed elsewhere as well (K. S. Andrews, D. Turner, *Optimization of the rate of sample injection in high-performance liquid chromatography with microsyringes and with sampling valves*. *Chromatographia*, **16** (1982) 175-177).

A similar approach has been applied by Mincsovcics and coworkers in overpressured layer chromatographic (OPLC) separations (E. Mincsovcics, M. Manach, L. Kecskes, B. Tapa, D. Papillard, E. Tyihak: *Single- and Multi-channel OPLC Separation on Non-segmented Sorbent Bed Using Flowing Eluent Wall for Operating Segmentation*, *J. Liq. Chrom. Rel. Technol.* **26**(16), 2611-2627, 2003) and in Published US Patent Application No. 2003020834. In this so-called flowing eluent wall (FEW) technique, the sorbent layer has been detached into active and non-active parts regarding the separation. Into the non-active segments, only mobile phase is introduced, while for the active part, the mobile phase as well as the sample is admitted. By this way, the so-called edge effect experienced during OPLC separations can be decreased or eliminated.

The disadvantage of these methods resides in that the method requires complicated apparatus and conventional columns can be used according to the method only after major modifications. Furthermore, changes in the flow rate of the eluent introduced near the column wall, i. e. at the column or chromatoplate perimeter („curtain flow”, „flowing eluent wall”) and changes thereof can significantly influence the performance of the chromatographic separation, especially efficiency.

SUMMARY OF THE INVENTION

On the basis of the state of the art discussed above, it can be concluded that there is a need for methods suitable for compensating the losses of performance in column chromatographic separations resulting from the radial inhomogeneity of the stationary phase residing in the chromatographic column, since such losses deteriorate the performance of the whole of the chromatographic system (made up by stationary phase, mobile phase, sample constituents, temperature). However, it has been found that the state of the art is silent about a method or apparatus, which is suitable and could be easily used for preventing or decreasing such a loss of separation performance.

The objective of our research-development activity was to develop a method and an apparatus which provides for preventing or decreasing the loss of performance in column chromatographic separations resulting from radial inhomogeneity and wall-effect of the chromatographic column. Said loss of performance is primarily manifested in the decrease of efficiency and resolution. The aim of our work was to provide an arrangement of a

chromatographic column which can be easily and robustly applied in existing column chromatographic separation systems known from the state of the art.

The above objective is achieved by the method and apparatus according to the present invention.

Surprisingly we have found that in the case when a part of the mobile phase eluted from the stationary phase exiting from the chromatographic column in the proximity of the wall of said column is directed separately from the part of the mobile phase eluted in the central cross section of the chromatographic column and only the portion of the mobile phase eluting in the central section of the chromatographic column close to the longitudinal symmetry axis is directed into a detector or into a sample collector, significant improvement in the performance of the chromatographic separating system can be achieved. This improvement manifests itself primarily by an increase of efficiency and resolution.

Furthermore, it has also been surprisingly found that by applying the method described above in analytical column chromatography, in certain cases, the improvement of detection limit can be observed.

During a preparative chromatographic run, by applying the method disclosed above, the separated components can be obtained in greater purity than by using methods according to the state of the art.

The part of the mobile phase exiting from the chromatographic column near the column wall can either be discarded or recycled.

SHORT DESCRIPTION OF THE DRAWINGS

Figure 1 demonstrates the parts of the column chromatographic system pertinent to the present invention.

Figure 2 demonstrates an embodiment of the apparatus according to the present invention wherein the means for directing the mobile phase eluting in the proximity of the column wall and diverting such stream from the central column outlet port are comprised in the outlet fitting element 3 of the chromatographic column.

Figure 3 demonstrates an embodiment of the apparatus according to the present invention wherein the means for directing the mobile phase eluting in the proximity of the column wall and diverting said stream are comprised in an insert 14.

Figure 4 demonstrates an embodiment of the apparatus according to the present invention wherein the means for directing the mobile phase eluting in the proximity of the column wall are comprised in a filter 9 retaining the stationary phase.

DETAILED DESCRIPTION OF THE INVENTION

According to the first object of the present invention, there is provided a method for improving the performance of column chromatographic separations, which comprises transferring a sample into a chromatographic mobile phase and separating sample components in known manner and subsequent to separation, separating and removing the part of the mobile phase eluting from the chromatographic stationary phase in the proximity of the wall of the chromatographic column and detecting, isolating and/or

further separating sample components eluted in the portion of the mobile phase eluting from the central part of the radial cross-section of the stationary phase perpendicular to the longitudinal axis thereof. Separation of the mobile phase stream into a part eluted from the proximity of the column wall and a second part eluted from the central axial section of the sorbent is preferably carried out at the exit cross section of said stationary phase.

The part of the mobile phase exiting near the column wall or in the central section of the exit cross-section of the stationary phase is diverted at the end of the stationary phase, conducted out separately and discarded or recycled. The outlet port located in the central (axial) part of the column end cross section provides for delivering the mobile phase containing analytes eluted from the more orderly structured central part of the stationary phase into a detector or for further separation or isolation. The central exit outlet of the chromatographic column prepared according to the present invention can be formed in a manner essentially known from the prior art.

The outlet comprising means for conducting the portion of the mobile phase exiting in the proximity of the column wall can be arranged in the form of one or more grommets, annular channels, recesses or grooves which are interconnected to an exit connecting port. The position of the outlet means can be determined according to the column diameter, method of column filling or column preparation and the field of application (i. e. analytical or preparative separation). The position and actual arrangement of the outlet port intended for conducting the portion of the mobile phase eluting near the column wall can be modified according to the application area of the chromatographic column as well. In general, however, the central outlet and

the outlet intended for diverting the portion of the mobile phase eluted near the column wall are arranged in the proximity of the contact surface of the stationary phase and the outlet fitting element for collecting the mobile phase exiting from the stationary phase. Accordingly, the location of the central outlet port is in the axis of the chromatographic column (i. e. the central part of the stationary phase), while grommets, annular channels, grooves or recesses collecting the part of the mobile phase exiting from the stationary phase bed near the column wall are connected to a port arranged off-axis, near the column wall. The means intended for conducting the mobile phase can contain additional channels to the annular or concentric liquid collecting channels in order to collect and conduct mobile phase exiting from different radial positions of the stationary phase cross-section perpendicular to the axis of the stationary phase. It is important, however, that the means intended for diverting the portion of the mobile phase, should not cause any flow disturbance, i. e. change of linear velocity profile near the column wall region. Such changes are not desired since they usually result in distortion of band shape and loss of performance.

During the use of the method according to the present invention, it is of great importance that the ratio of the flow rate of the eluent stream entering into the detector and that of the eluent stream entering into the chromatographic column (i.e. delivered by the high-pressure pump) is kept precisely constant. In the case when the above-mentioned ratio exhibits fluctuations, linearity of detection as well as efficacy/resolution of the separation may suffer.

The above objective can be achieved by maintaining the flow rate of the

diverted eluent stream exiting from the chromatographic column in the proximity of the column wall strictly constant. It has been found that in case of analytical separations, it is sufficient to install a section of tubing (i.e. capillary tube) providing a constant pressure resistance (pressure drop). Pressure drop in chromatographic tubing (i.e. capillaries) depends on the material of said tubing, composition and temperature of the mobile phase, the inner diameter and the length of the tubing which is available from the state of the art, i.e. from commercial vendors or can be determined experimentally using techniques known from the prior art.

In some cases, such as during preparative separations, the need might arise for installing a flow or pressure controller into the stream of the mobile phase eluted and exiting nearby the column wall in order to maintain the flow rate constant. Such controllers are known from the state of the art and these instruments are available commercially.

The advantage of the method according to the present invention resides in the fact that said method does not require an auxiliary mobile phase stream, thus there is no need for modifying the mobile phase delivery system and usually no excess mobile phase consumption occurs. The modification of the chromatographic column is minor and involves the outlet (exit) fitting element or some related smaller parts only (Figure 1). The modified outlet element, filter or insert can be machined in a simple way according to methods known from the state of the art, e.g. using computer-controlled (CNC) machining. A further advantage of the solution according to the present invention resides in the fact that the flow rate of the mobile phase stream diverted from the outlet fitting element 3 can be simply controlled by

changing the pressure drop of the exit tubing (i.e. installing a section of tubing having known pressure drop) or by choking.

The method according to the present invention results in improved performance of the chromatographic system. By performance, the separating ability of the system is meant, which is inversely proportional with the degree band broadening. Basically, any methods known for the assessment of resolution or efficacy of a chromatographic system can be used. For example, the efficacy or the height equivalent to a theoretical plate (HETP) can be calculated for each peak (i. e. each sample component). In case of critical pairs, the resolution can also be calculated. Methods for such calculations are known from the state of the art.

According to a further aspect of the present invention, there is provided an apparatus for carrying out the method according to the invention. The scheme of the apparatus is demonstrated by Figure 1, which is principally a chromatographic column containing a stationary phase with modified outlet fitting element 3 according to the present invention. Chromatographic mobile phase is delivered at a constant flow rate by a high-pressure pump according to methods known from the state of the art. The method according to the present invention can be used during isocratic as well as gradient elution.

The sample to be separated can be injected into the stream of the mobile phase using any manual or automatic sample injector known from the prior art. The mobile phase stream exiting the sample injector enters the chromatographic column through connection tubing 4 and inlet fitting

element 2. The mobile phase stream exits the chromatographic column through outlet fitting element 3. The inlet of the chromatographic column 1 comprises the inlet fitting element 2, whereby the connection tubing 4 and the chromatographic column 1 are attached without dead volume by a pressure-tight seal and by means suitable for retaining the stationary phase. Any inlet fitting element 2 and fitting chromatographic column 1 known from the state of the art are suitable for use in the method according to the present invention after slight modification. Inlet and outlet fitting elements (2,3) are usually attached to the chromatographic column by threads and compression fittings. Nevertheless, any other method for pressure-tight connection known from the state of the art, e.g. flanged fittings can be used provided that pressure-tight and low or zero dead-volume connections can be established. Connections referred to as ports in the present specification are apertures by which the mobile phase enters or leaves the apparatus. Ports are usually provided with means for establishing pressure-tight and low or zero dead volume connection between the parts of the apparatus, such as a chromatographic column according to the present invention and the interconnecting tubing. As an example, such connections can be established by compression screws and conical sealing rings (ferrules).

The method according to the present invention can be carried out using a chromatographic column equipped with an outlet fitting element 3 known from the prior art with slight modifications. Thereby a further aspect of the present invention related to the configuration of the outlet element 3 is provided, wherein an outlet element 3 known from the state of the art having a central outlet exit port is provided with means suitable for collecting and conducting the portion of the mobile phase exiting from the

chromatographic column 1 in the proximity of the column wall and attached to a second outlet port 6 by a suitable connector. Means for collecting and conducting the portion of the mobile phase exiting from the chromatographic column 1 in the proximity of the column wall can be shaped as one or more grommets, annular or concentric grooves, recesses or channels, which are interconnected by suitable means, e.g. by channels or bores to one or more outlet ports 6. The mobile phase stream exiting the chromatographic column 1 centrally i.e. collaterally to the axis of the column is directed through the connection tubing 5 into a detector or alternatively, for subsequent separation of fractionation. The mobile phase stream exiting the chromatographic column in the proximity of the column wall exits the outlet fitting element 3 through port and connection tubing 6, whereto optionally a flow or pressure controller can be attached.

Preferable forms of the means for collecting and conducting the portion of the mobile phase exiting from the chromatographic column 1 in the proximity of the column wall are annular grooves or channels interconnected to one or more outlet ports 6. There is no practical limitation regarding the position or cross-sectional profile of such grooves or channels. The preferable cross-section profile is half-circular. Other suitable cross-sectional profiles advantageous from the point of the view of easy and convenient manufacturing are rectangular, triangular or oval (half-elliptical).

It has been found that it is advantageous that the width of the annular grooves or channels for collecting and conduction the mobile phase portion leaving the chromatographic column near the column wall is chosen to 0.5 to 40 percent of the inner diameter of the chromatographic column. A

particularly preferably range for this width is 5 to 8 percent of the column inner diameter. In case of rectangular, triangular or half-elliptical cross-section, the depth of the channel is 10 to 500 percent of the width thereof.

In Figure 2, the most advantageous embodiment of an outlet fitting element 3 is depicted. In the left longitudinal section drawing, central port and connection tubing 5 as well as port and connection tubing for conducting the mobile phase exiting near the column wall 6 are attached to outlet element 3 by compression ferrule and screw. Outlet element 3 is attached to a chromatographic column 1 comprising the chromatographic tube 7 and the stationary phase 8 containing therein e.g. in the form of a sorbent bed, are connected with a threaded connection (not shown). Between the chromatographic tube 7 and the outlet element 3, a retaining filter 9 is arranged, which is, upon closure of the threaded connection, fringed upon the surface of the stationary phase 8 and the outlet element 3 without any side or circumferential dead volume or clearance. Onto the inner surface of the outlet element 3 facing to the retaining filter 9, an annular groove 10 suitable for collecting the mobile phase is located at a distance smaller than the inner diameter of the chromatographic tube 7 measured from the bore 11 connected to the central outlet port 5, said annular groove 10 communicating to the port 6 through the channel 12. The cross-sectional view of the annular groove 10 at the contacting plane of the filter 9 and outlet element 3 is shown on the right hand side cross-sectional view „A” of Figure 2, wherein the annular groove 10, outlet port 6 and communicating channel 12 are demonstrated together with a compression screw and sealing ring. Although the embodiments in the present application are demonstrated by fittings comprising a compression crew and a sealing ring (ferrule), a

person skilled in the art would equally well appreciate other means known from the prior art suitable for providing low dead-volume pressure-tight fittings.

The embodiment of the present invention demonstrated in Figure 2 can be advantageously used in case of chromatographic columns filled with a bed of granular stationary phase, especially on an analytical scale. However, there is no limitation to the quality, particle size, adsorption properties, chemical compositions of the stationary phase used in the apparatus according to the present invention. Thus, a granular or porous block polymer stationary phase of any desirable chemistry can be equally well used.

According to a further embodiment of the present invention depicted in Figure 3, the stationary phase 8 is secured with an insert 13 located inside the outlet fitting element 3 said insert 13 having at least one annular groove 14 on the surface facing towards the stationary phase 8 or towards an optional retaining filter element 9 placed onto the surface of the stationary phase 8. Said annular groove 14 communicates through a channel 12 with a port 15 in order to divert the mobile phase stream exiting from the chromatographic column in the proximity of the column wall. The mobile phase stream exiting from the central port 16 of the outlet element 3 and the bore 11 attached thereto flows through a connection tubing into the detector.

According to a still further embodiment of the present invention depicted in Figure 4, the mobile phase collecting annular groove or channel 17 for collecting the mobile phase stream exiting from the chromatographic

stationary phase in the proximity of the column wall is formed on the surface of a filter element 9 retaining and sealing the chromatographic stationary phase.

According to a further embodiment of the present invention, a porous block polymer stationary phase is used and the annular groove or channel is formed directly on the exit surface of said „monolithic” block polymer stationary phase provided that the outlet element 3 possesses adequately arranged ports. This embodiment is demonstrated in principle in Figure 4 with the modification that the annular collecting groove or channel 17 is formed directly on the surface of the block polymer stationary phase (dotted area) instead of the filter element 9. When a block polymer type stationary phase is used, the embodiments shown in Figures 2 and 3 can be used as well with the modification that no retaining filter 9 is necessary.

The method and apparatus according to the present invention can be used for any column chromatographic separation system or stationary phase (e.g. normal, reversed phase; ion exchange, size exclusion chromatography; affinity chromatography, hydrophobic interaction chromatography etc.). It has been found that the method and apparatus according to the present invention can be applied for improving the performance of the chromatographic system not only during analytical but in preparative separations as well even in the case of traditional column when the stationary phase has relative greater particle size (e.g. 63-200 μm). The use of the method and apparatus is not restricted to the field of elution chromatography; it can be equally well used in displacement and frontal chromatographic separations for improving separation performance as well.

The effects of the method and apparatus are demonstrated by the following example. In an isocratic reversed phase high performance separation, 250 mm long, 4.6 mm i.d. columns filled with octadecyl-silica stationary phase were tested with a four-component test mixture. In the first series of tests (series „A”), the column equipped with an outlet element 3 identical to inlet element 2 was used. Thus, in this case, no annular groove or channel attached to port 6 for diverting the mobile phase exiting from the column near the column wall was present in outlet element 3. The total eluent stream flowed into the detector having a detector cell volume of 5 μ l. In the second test series (series „B”), the column was equipped with an outlet element 3 containing an insert according to the present invention (Figure 3) having a groove of rectangular cross section having 0.5 mm width and 0.5 mm depth located directly at the column wall and the flow rate of the mobile phase exiting the chromatographic column centrally and flowing into the detector was set to 23.5 % of the total flow by means of a flow restricting capillary attached to port 6. The detector cell volume was the same as in series „A”. In series „A”, the efficacy (number of theoretical plates) for the sample components was 11869, 11627, 11190 and 10727, respectively. In series „B”, the efficacy (number of theoretical plates) was 13010, 12870, 12850 and 12569, respectively. Thus, the efficacy improved by a value of 9 to 15 %. In the test series „C”, the flow rate entering into a detector cell of 0.5 μ l was 7 % of the total flow rate, the number of theoretical plates for the same test compounds were 14720 (24%), 14024 (20,6%), 12743 (13,88%) and 12301 (14,67%), respectively, thus the improvement in the number of theoretical plates was between 14 and 24 %.

We claim:

1. Method for improving the performance of column chromatographic separations and systems, which comprises introducing a sample to be separated into a mobile phase stream and separating sample constituents in a known manner and subsequently to chromatographic separation, separating and discarding the portion of the mobile phase exiting from the chromatographic stationary phase near the column wall while simultaneously subjecting the stream constituted from the portion of the mobile phase exiting from the central axial part of said chromatographic stationary phase to analyte detection, fractionation or further separation.
2. Method according to claim 1, characterized in that the portions of the mobile phase eluting near the wall of the chromatographic column and in the central section of said column are separated at the end surface of the stationary phase.
3. Method according to claim 1 or claim 2, characterized in that the efficiency or resolution is improved.
4. Method for reducing or preventing the loss of efficiency in chromatographic columns and reducing band broadening resulting from radial stationary phase inhomogeneity, which comprises introducing a sample to be separated into a mobile phase stream, separating the constituents of said sample in a known manner and subsequently to chromatographic separation, separating and discarding the portion of the mobile phase exiting from the chromatographic column near the column wall while simultaneously subjecting a stream constituted from the portion of the mobile phase exiting from the central part of said chromatographic column to

analyte detection, fractionation or further separation.

5. Apparatus demonstrated on Figure 1 for performing the method according to any of claims 1 to 4, which comprises inlet means 2, chromatographic column 1 and outlet means 3 wherein the outlet means 3 contains a central port 5 and one or more ports 6 suitable for collecting and discarding the mobile phase exiting from the chromatographic column 1 near the column wall.
6. Apparatus according to claim 5, characterized by that the outlet element 3 according to Figure 2 comprises central outlet port 5, 11 and outlet port for the mobile phase exiting in the proximity of the column wall 6, 12, wherein the outlet port 5 is connected to a central axial bore 11 and the outlet port 6,12 is connected to annular eluent collecting grommets, groove, recess or channel 10, preferable a groove situated on the surface of the outlet element 3 facing the chromatographic stationary phase 8 at a distance smaller than the half of the inner diameter of the chromatographic tube 7 from the axis of the central exit bore 11.
7. Apparatus according to claim 5, characterized by that the outlet element 3 demonstrated on Figure 3 contains an insert 13, said insert 13 comprising one or more, preferably annular grommets, groove, recess or channel 14, preferably a groove on the surface of the insert 13 facing the stationary phase 8 or the optional filter element 9 positioned on the surface of said stationary phase 8 and wherein said one or more grommets, groove, recess or channel 14 are interconnected to the port 6,12 by one or more eluent connection bores 15 and wherein a central connecting bore 16 is connected to a central port 5, 11.
8. Apparatus according to claim 5, characterized by that the outlet

element 3 demonstrated in Figure 4 comprises outlet ports 5 and 6 and a filter 9 positioned on the surface of the stationary phase 9, wherein outlet port 5 communicates with the axial bore 11, said filter element 9 is provided with annular mobile phase collecting grommets, groove, recess or channel 17, preferably a groove and means 12 in outlet fitting element 3 for connecting said grommets, groove, recess or channel 17 to ports 6, 12.

9. Apparatus according to claim 5, comprising a block polymer stationary phase inside the chromatographic column 1 wherein a mobile phase collecting annular groove or channel 10 is provided on the surface of the stationary phase 8 at a distance smaller than the half inner diameter of the chromatographic tube 7 from the axis of the central outlet bore 5, 11 and an outlet element 3 comprising a port 6, 12 in a way that after assembling the chromatographic column 1 and the outlet element 3, the mobile phase collecting annular groove or channel 10 provided on the surface of the block polymer stationary phase 8 is aligned with the port 6, 12 located on the outlet element 3.

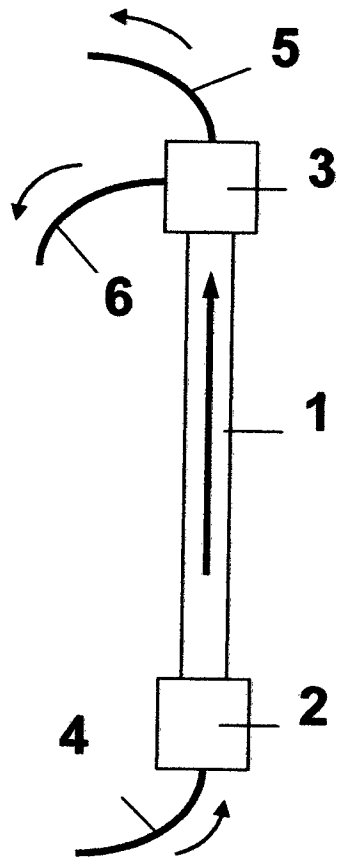


Figure 1

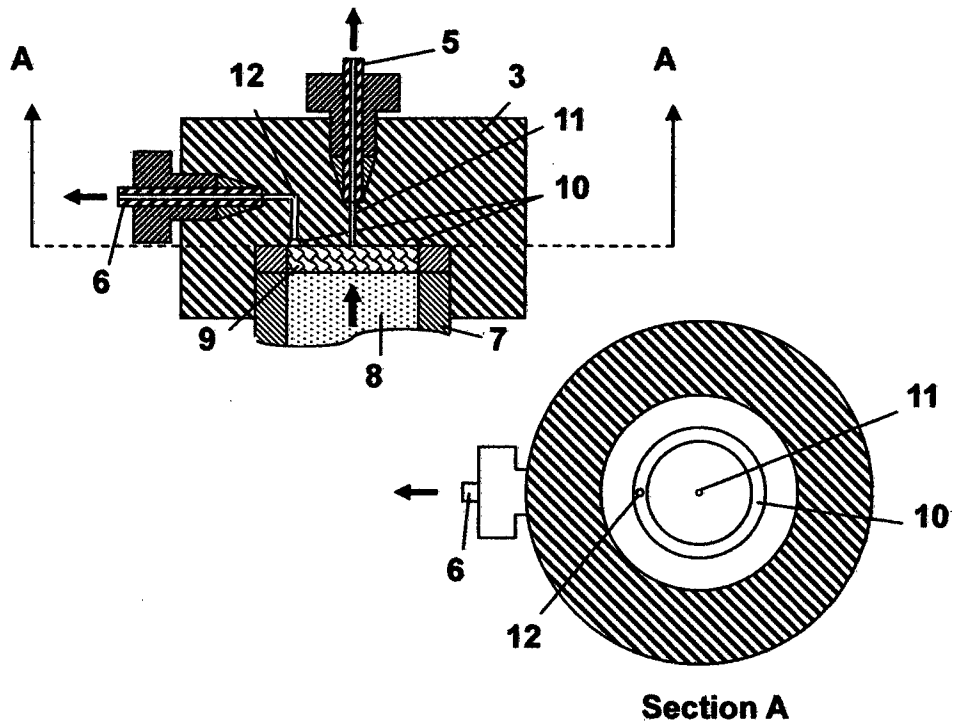


Figure 2

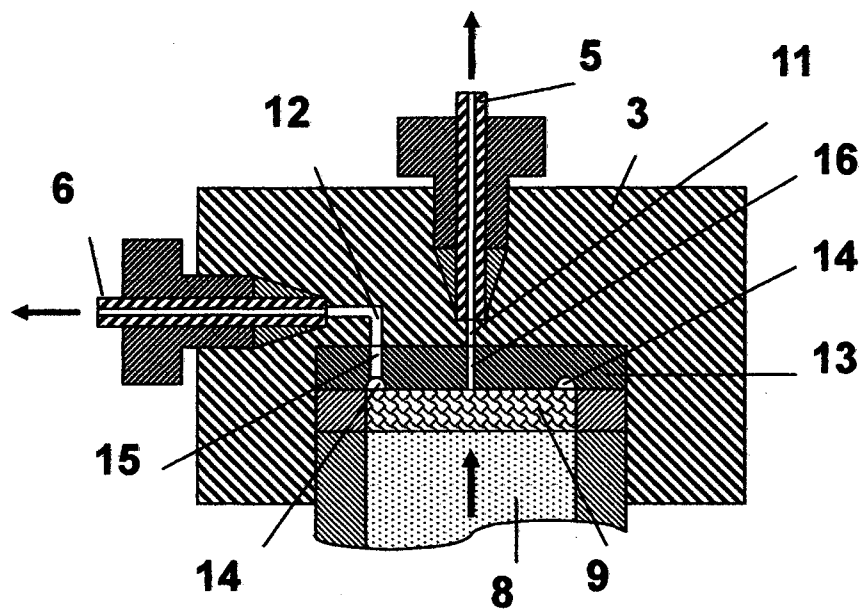


Figure 3

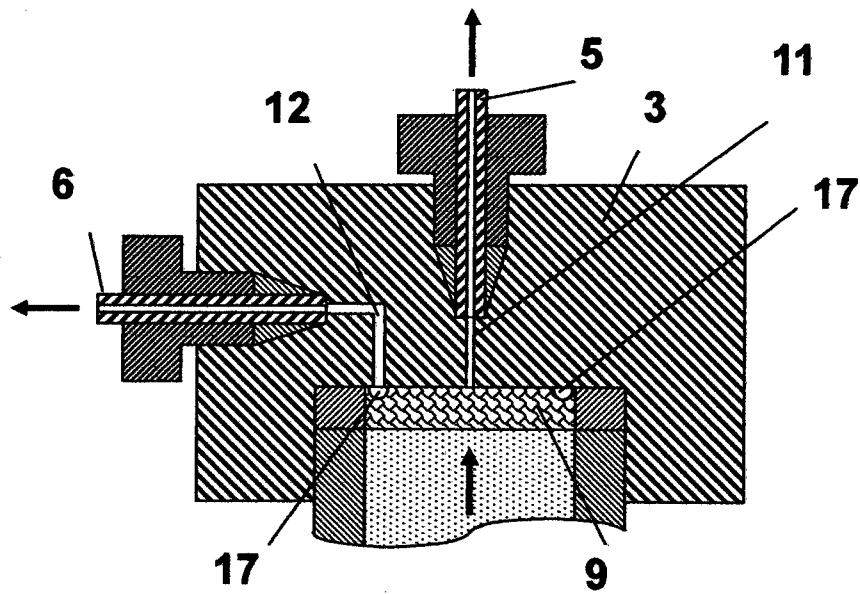


Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2012/000049

A. CLASSIFICATION OF SUBJECT MATTER

INV. B01D15/22 B01D15/24 G01N30/60
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

B01D G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP 62 240857 A (KURITA WATER IND LTD) 21 October 1987 (1987-10-21) abstract; figures -----	1-9
X	EP 0 257 582 A2 (CESKOSLOVENSKA AKADEMIE VED [CS]) 2 March 1988 (1988-03-02) claims; figures 1,2 -----	5
A,P	WO 2011/159232 A1 (GE HEALTHCARE BIO SCIENCES AB [SE]; BELLQVIST PETER [SE]; GEBAUER KLAU) 22 December 2011 (2011-12-22) page 6, line 14 - page 8, line 5; figures -----	1-9
A	WO 03/101581 A1 (AMERSHAM BIOSCIENCES AB [SE]; GEBAUER KLAUS [SE]) 11 December 2003 (2003-12-11) page 6, line 1 - page 8, line 15; figures ----- -/--	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search

4 October 2012

Date of mailing of the international search report

15/10/2012

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Fourgeaud, Damien

INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2012/000049

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 104 128 A2 (MOTT LAMBERT H) 28 March 1984 (1984-03-28) page 6, line 31 - page 9, line 27; figures 1-3	1-9
A	----- JP 2000 088829 A (SHIMADZU CORP) 31 March 2000 (2000-03-31) abstract; figures -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/HU2012/000049

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
JP 62240857	A	21-10-1987	NONE	
EP 0257582	A2	02-03-1988	CS 8606128 A1 EP 0257582 A2 JP 63101748 A US 4752391 A	15-02-1988 02-03-1988 06-05-1988 21-06-1988
WO 2011159232	A1	22-12-2011	NONE	
WO 03101581	A1	11-12-2003	AT 367191 T AU 2003242602 A1 DE 60315021 T2 EP 1509303 A1 ES 2288619 T3 JP 4278612 B2 JP 2005527831 A US 2005236312 A1 WO 03101581 A1	15-08-2007 19-12-2003 20-03-2008 02-03-2005 16-01-2008 17-06-2009 15-09-2005 27-10-2005 11-12-2003
EP 0104128	A2	28-03-1984	AU 556722 B2 AU 1483083 A CA 1192848 A1 DE 3370075 D1 EP 0104128 A2 JP 59079158 A US 4399032 A	13-11-1986 29-03-1984 03-09-1985 09-04-1987 28-03-1984 08-05-1984 16-08-1983
JP 2000088829	A	31-03-2000	JP 3975575 B2 JP 2000088829 A	12-09-2007 31-03-2000