(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 2 626 697 A1**

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
14.08.2013 Bulletin 2013/33

(51) Int Cl.:
G01N 30/32 *(2006.01)* ***G01N 30/20*** *(2006.01)*

(21) Application number: **12154228.6**

(22) Date of filing: **07.02.2012**

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME

(71) Applicant: **King Saud University**
11421 Riyadh (SA)

(72) Inventors:
- **Al-Warthan, Abdulrahman Abdullah**
  11333 Riyadh (SA)
- **Hadj Ahmed, Ahmed Yacine Badjah**
  Riyadh 11333 (SA)
- **Al-Othman, Zeid Abdullah**
  Riyadh 11333 (SA)
- **Aqel, Ahmad**
  Riyadh 11333 (SA)

(74) Representative: **Goddar, Heinz J.**
Boehmert & Boehmert
Pettenkoferstrasse 20-22
80336 München (DE)

Remarks:
Amended claims in accordance with Rule 137(2) EPC.

(54) **Liquid chromatography device**

(57) The present invention relates to a liquid chromatography device comprising a pump (1), a sample injector (2), a splitting device (3), a restrictor (4), and a liquid chromatography column (5), wherein the pump (1), the sample injector (2), the splitting device (3) and the liquid chromatography column (5) were connected in series; the sample injector (2) and the splitting device (3) are placed between the pump (1) and the liquid chromatography column (5); and the splitting device (3) is further connected with the restrictor (4).

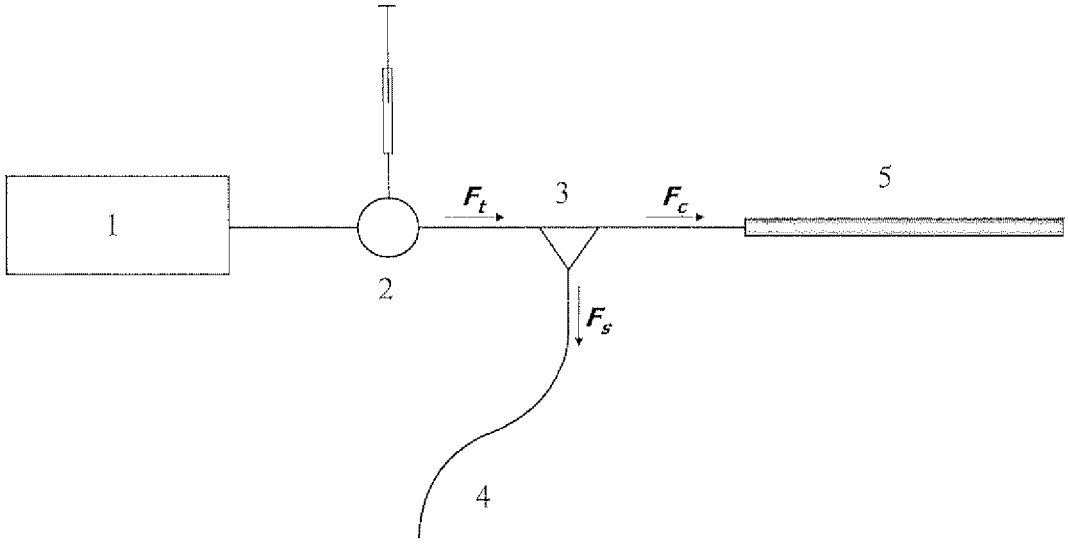

Fig. 1

EP 2 626 697 A1

Description

[0001] The present invention relates to a liquid chromatography device.

[0002] The term liquid chromatography (LC) covers various techniques which are useful analytical tools for separation, identification and determination of a wide range of compounds and various simple or complex mixtures. During the recent years, capillary liquid chromatography has emerged as a promising micro-separation technique that combines high efficiency and system miniaturization.

[0003] In capillary LC columns having smaller internal diameters than conventional packed HPLC columns are used. For a given injection sample volume, a smaller column diameter gives higher peaks which lead to a better detection limit. Thus, a capillary column with a 0.1 mm inner diameter corresponds to a sensitivity more than 2000 times higher compared to a conventional 4.6 mm packed column.

[0004] The volume of the injected sample is another important parameter which can affect both the sensitivity and the column performance. Most recent capillary LC instruments are equipped with nano-injectors allowing injection of sample volumes in the nanoliter range. However, commercial nano-injectors have a fixed volume which can not be changed as with conventional injectors which are equipped with a changeable sample loop.

[0005] US 6,289,914 B1 relates to a micro splitter in flow systems especially used for separation techniques in analytical chemistry such as microliquid chromatography, high pressure liquid chromatography and ancillary techniques. The splitter system comprises a micro splitter and a micro mixer which are connected by a microbore tubing.

[0006] US 7,465,382 B2 discloses a precision flow controller which is capable of providing a flow rate less than 100 microliters/minute and varying the flow rate in a prescribed manner where the accuracy and precision of the flow rate is less than 5% of the flow rate.

[0007] US 2009/0165873 A1 relates to a splitter for a pressurized primary flow stream of a mobile phase. The splitter comprises a first splitting stage that divides the primary flow stream between a major split flow stream and a minor split flow stream and a second splitting stage that divides the diluted minor flow stream.

[0008] US 2007/0283746 A1 discloses an injection device including a carrier inlet, a sample inlet, a waste outlet and a chamber outlet attached to a separation column. Valves are used to control flow such that the sample flows into chamber and is carried into the chamber outlet.

[0009] It is an object of the present invention to provide a liquid chromatography device which overcomes the drawbacks of the prior art. Particularly, a liquid chromatography device shall be provided which allows to reduce and control simultaneously both the mobile phase flow rate as well as the volume of the injected sample. Further, it is an object of the present invention to provide a liquid chromatography device to reduce and control the column flow rate and the sample volume in a wide range as well as to fine control the sample volume in an easy, accurate, reproducible and cost efficient way.

[0010] These objects are achieved by a liquid chromatography device comprising:

a) a pump;

b) a sample injector;

c) a splitting device;

d) a restrictor ; and

e) a liquid chromatography column and detection and analyzing unit;

wherein the pump, the sample injector, the splitting device and the liquid chromatography column are connected in series, the sample injector and the splitting device are placed between the pump and the liquid chromatography column; and the splitting device is further connected with the restrictor.

[0011] In a preferred embodiment, the splitting device is placed behind the sample injector.

[0012] Even preferred, the pump is a high performance liquid chromatography (HPLC) pump.

[0013] In a further preferred embodiment, the sample injector is a six-pon injector equipped with a fixed volume sample loop.

[0014] Preferred, the splitting device is a splitting tee.

[0015] In one embodiment, the splitting tee is a zero dead volume tee.

[0016] In another embodiment, the restrictor is a tubing.

[0017] Preferably, the tubing is a capillary tubing.

[0018] Even preferred, the liquid chromatography column is a capillary liquid chromatography column.

[0019] Preferably, the splitting device and the restrictor are made of stainless steel or polyether ether ketone (PEEK).

[0020] Even preferred, the length of the tubing is in a range from 0.1 to 1.0 mm, preferably is in a range from 1 to 5 mm, most preferably is in a range from 2 to 4 mm, and the diameter of the tubing is in a range from 10 to 500 μm, preferably is in a range from 100 to 200 μm.

[0021] Also preferred, the length of the liquid chromatography column is in a range from 10 to 500 mm, preferably is in a range from 50 to 200 mm, and the diameter of the liquid chromatography column 5 is in a range from 100 to 500 μm, preferably is in a range from 300 to 400 μm.

[0022] Preferably, the connections between the pump, the sample injector, the splitting device, the restrictor and the liquid chromatography column are made by capillary tubes and the fittings of the connections are made of PEEK.

[0023] In a most preferred embodiment, the liquid chromatography device according to the present invention consists of a pump, a sample injector, a splitting device, a restrictor, a liquid chromatography column and detection and analyzing unit. Any detection and analyzing unit known in the art can be taken for the inventive liquid chromatography device.

[0024] According to the invention is also the use of the inventive liquid chromatography device for generating very small sample values having a volume in a range from 1 nl to 20 μl, preferably having a volume in a range from 1 to 300 nL, most preferably having a volume in a range from 1 to 50 nL.

[0025] Surprisingly, it was found that the inventive liquid chromatography device solves the problems by providing an easy and cost-efficient way to reduce and control the mobile phase flow rate as well as the sample injection volume in liquid chromatography processes in a wide range from several micro liters/min to a few nano liters/min.

[0026] Further characteristics and advantages of the invention result from the detailed description of the preferred embodiment particularly in context with the example and the attached drawings, wherein

Fig. 1 shows a schematic illustration of an embodiment of the liquid chromatography device according to the invention.

Fig. 2 shows the relation between total flow F_t and column flow F_c for an inventive device.

Reference is now made to Fig. 1 which illustrates the liquid chromatography device and its components, according to an embodiment of the invention. The inventive liquid chromatography device comprises a pump 1, a sample injector 2, a splitting device 3, a restrictor 4 and a liquid chromatography column 5.

[0027] Any pumps suitable to provide a constant or variable liquid flow in liquid chromatography can be used as pump 1 of the inventive liquid chromatography device, e.g. a conventional HPEC pump with a flow rate in the range from 0.01 to 10 mL/min. The pump 1 is in direct connection with the sample injector 2, whereby the connection is made by an appropriate tubing, for example by a capillary tubing. The fittings connecting the appropriate tubing with the pump 1 and the sample injector 2 are made of a material allowing working under pressure. Particularly, PEEK can be advantageously used as fitting material due to its highly inert and resistant properties.

[0028] A sample to be be analyzed can be provided in the sample injector using conventional methods, for example by injection of the sample using a syringe. The sample injector 2 is in connection with the splitting device 3. Connections are again made by using tubing and fitting devices as mentioned above.

[0029] The splitting device 3 consists of a simple tee-fitting made of stainless steal or PEEK with three openings for making connection with the other components of the inventive liquid chromatography device. The splitting device 3 should have the lowest internal volume possible (known as "zero dead volume") to avoid sample dilution and unnecessary PEEK broadening. Using a splitting device made of PEEK allows working with pressures up to 5.000 psi. If it is intended to operate under higher pressures up to 10.000 psi, stainless steal would be the splitting device 3 material of choice.

[0030] One of the remaining openings of the splitting tee is used to make contact to the restrictor 4. A tubing can be used as restrictor. By choosing the length and the inner diameter of the tubing, the mobile phase flow rate and the volume of the injected sample can be manipulated as discussed in detail below.

[0031] By means of the remaining third opening of the splitting tee, contact is made to the liquid chromatography column 5. In combination with the inventive liquid chromatography device an appropriate sensor or analyzer, for example in case of analyzing aromatic or colored substances an UV/Vis detector, can be attached to the inventive device behind the liquid chromatography column. 5.

[0032] By using the inventive liquid chromatography device, the mobile phase with a total flow rate (F_t) is split into two parts, the column flow F_c through the liquid chromatography column 5 and the split flow (F_s) through the restrictor 4. The split ratio R represents the total flow to column flow ration:

$$R = F_t / F_c$$

[0033] On the one hand, the split ratio R depends on the liquid chromatography column 5 characteristics (length, diameter, porosity, etc.). Besides this, it can notablely be adjusted by changing the length and/or the inner diameter of the restrictor 4. On the other hand, by using a constant split ratio R the mobile phase flow rate through the liquid chromatography column 5 F_c can be very simply controlled by adjusting the total flow rate F_t at the pump 1. Thus, the flow rate through the liquid chromatography column 5 F_c can be accurately adjusted in a very wide range from several mieroliters/min down to a few nanoliters/min.

[0034] Another critical issue of the invention is to provide the possibility to control the sample injection volume in capillary chromatography. After injection of the sample through the sample injector 2 it is split with the mobile phase in the same ratio R. This derivation technique allows injection of very small amounts of sample to the liquid chromatography column 5 while the remaining sample volume is flushed through the restrictor 4. Subsequently, the mobile phase excess flowing through the restrictor 4 can be sent back to the solvent reservoir, in case of isocratic LC operation. This option contributes to

a drastically reduction of solvent consumption.

[0035] In conclusion, it can be noted that the longer the restrictor or the lower its inner diameter, the lower will be the split flow F_s and the higher the split ratio R. Thus, when using the same restrictor 4, the R to F_c ratio remains constant, as shown in Fig. 2.

[0036] Another interesting option according to the present invention is to place the splitting device 3 between the pump 1 and the sample injector 2. This allows to split only the mobile phase flow without effecting the injected sample volume which corresponds therefore to the entire sample loop volume. This option permits a full loop injection of the sample without affecting either the column flow rate or the retention time.

[0037] A specific example for the use of the liquid chromatography device of the present invention is given in the following in order to illustrate the invention without limiting its scope in any way.

[0038] A capillary monolithic column 5 of the dimensions 100mm x 0.320mm i.d was used. The mobile phase consisted of an acetonitrile/water mixture (50:50, v/v) with a total flow rate set at the pump 1 of 0.6 mL/min, The sample injector 2 was equipped with a sample loop made of PEEK tubing (71 mm length, 0.127 mm i.d.). The corresponding volume of this loop was 900 nL. As model analyte, a toluene solution (concentration 0.04% in acetonitrile) was injected with UV detection at a wavelength of 260 nm. The splitting tee 3 was placed between the sample injector 2 and the capillary column 5, as it can be seen in Fig. 1. The restrictor 4 was a PEEK tube with a length of 3 m and an inner diameter of 0.127 mm.

[0039] Under this conditions, the total flow F_t was 0.6 mL/min while the column flow measured accurately at the column outlet F_c was 3 μL/min. The corresponding split ratio is:

$$R = F_t/F_c = 0.6/0.003 = 200$$

[0040] This means that the sample volume injected through the column is split in the same ratio and corresponds to:

$$V_{inj} = 900 / 200 = 4.5\text{nL}.$$

[0041] To assess the accuracy and reproducibility of the presented method, several experiments were carried out by modifying the total flow rate at the pump and measuring the actual mobile phase flow rate at the capillary column outlet. The obtained values are presented in Fig. 2.

[0042] The total flow rate ranged from 0.1 to 2.0mL/min while the corresponding measured column flow rate was in the range 0.5 to 10μL/min. Fig. 2 shows that the relationship is linear in the investigated range with a correlation factor of 0.9988. The split ratio with a value of 200 corresponds to the curve slope and is constant on the whole range.

[0043] The features disclosed in the foregoing description, the claims and the accompanying drawings may, both separately and in any combination thereof, be material for realizing the invention in diverse forms thereof.

Claims

1. Liquid chromatography device comprising:

   a) a pump (1);
   b) a sample injector (2);
   c) a splitting device (3);
   d) a restrictor (4); and
   e) a liquid chromatograph column (5) and detection and analyzing unit;
   wherein the pump (1), the sample injector (2), the splitting device (3) and the liquid chromatography column (5) are connected in series; the sample injector (2) and the splitting device (3) are placed between the pump (1) and the liquid chromatography column (5); and the splitting device (3) is further connected with the restrictor (4).

2. Liquid chromatography device according to claim 1, wherein the splitting device (3) is placed behind the sample injector (2).

3. Liquid chromatography device according to claim 1 or 2, wherein the pump (1) is a high performance liquid chromatography (HPLC) pump.

4. Liquid chromatography device according to any of the preceding claims, wherein the sample injector (2) is a six-port injector equipped with a fixed volume sample loop.

5. Liquid chromatography device according to any of the preceding claims, wherein the splitting device (3) is a splitting tee.

6. Liquid chromatography device according to claim 5, wherein the splitting tee is a zero dead volume tee.

7. Liquid chromatography device according to any of the preceding claims, wherein the restrictor (4) is a tubing.

8. Liquid chromatography device according to claim 7, wherein the tubing is a capillary tubing.

9. Liquid chromatography device according to any of the preceding claims, wherein the liquid chromatog-

raphy column (5) is a capillary liquid chromatography column.

10. Liquid chromatography device according to any of the preceding claims, wherein the splitting device (3) and the restrictor (4) are made of stainless steel or polyether ether ketone (PEEK).

11. Liquid chromatography device according to claim 7 or 8, wherein the length of the tubing is in a range from 0.1 to 10 mm, preferably is in a range from 1 to 5 mm, most preferably is in a range from 2 to 4 mm, and the diameter of the tubing is in a range from 10 to 500 μm, preferably is in a range from 100 to 200 μm.

12. Liquid chromatography device according to any of the receding claims, wherein the length of the liquid chromatography column (5) is in a range from 10 to 500 mm, preferably is in a range from 50 to 200 mm, and the diameter of the liquid chromatography column (5) is in a range from 100 to 500 μm, preferably is in a range from 300 to 400 μm.

13. Liquid chromatography device according to any of the preceding claims, wherein the connections between the pump (1), the sample injector (2), the splitting device (3), the restrictor (4) and/or the liquid chromatography column (5) are made by capillary tubes and the fittings of the connections are made of PEEK.

14. Use of the liquid chromatography device according to any of the preceding claims for generating very small sample values having a volume in a range from 1 nl to 20 μl, preferably having a volume in a range from 1 to 300 nL, most preferably having a volume in a range from 1 to 50 nL.

Amended claims in accordance with Rule 137(2) EPC.

1. Liquid chromatography device comprising:

a) a pump (1);
b) a sample injector (2);
c) a splitting device (3);
d) a restrictor (4); and
e) a liquid chromatography column (5) and detection and analyzing unit;

wherein the pump (1), the sample injector (2), the splitting device (3) and the liquid chromatography column (5) are connected in series; the sample injector (2) and the splitting device (3) are placed between the pump (1) and the liquid chromatography column (5); and the splitting device (3) is further connected with the restrictor (4); **characterized in that** the splitting device (3) is placed behind the sample injector (2).

2. Liquid chromatography device according to claim 1, wherein the pump (1) is a high performance liquid chromatography (HPLC) pump.

3. Liquid chromatography device according to claims 1 or 2, wherein the sample injector (2) is a six-port injector equipped with a fixed volume sample loop.

4. Liquid chromatography device according to any of the preceding claims, wherein the splitting device (3) is a splitting tee.

5. Liquid chromatography device according to claim 4, wherein the splitting tee is a zero dead volume tee.

6. Liquid chromatography device according to any of the preceding claims, wherein the restrictor (4) is a tubing.

7. Liquid chromatography device according to claim 6, wherein the tubing is a capillary tubing.

8. Liquid chromatography device according to any of the preceding claims, wherein the liquid chromatography column (5) is a capillary liquid chromatography column.

9. Liquid chromatography device according to any of the preceding claims, wherein the splitting device (3) and the restrictor (4) are made of stainless steel or polyether ether ketone (PEEK).

10. Liquid chromatography device according to claim 6 or 7, wherein the length of the tubing is in a range from 0.1 to 10 mm, preferably is in a range from 1 to 5 mm, most preferably is in a range from 2 to 4 mm, and the diameter of the tubing is in a range from 10 to 500 μm, preferably is in a range from 100 to 200 μm.

11. Liquid chromatography device according to any of the preceding claims, wherein the length of the liquid chromatography column (5) is in a range from 10 to 500 mm, preferably is in a range from 50 to 200 mm, and the diameter of the liquid chromatography column (5) is in a range from 100 to 500 μm, preferably is in a range from 300 to 400 μm.

12. Liquid chromatography device according to any of the preceding claims, wherein the connections between the pump (1), the sample injector (2), the splitting device (3), the restrictor (4) and/or the liquid chromatography column (5) are made by capillary tubes and the fittings of the connections are made

of PEEK.

13. Use of the liquid chromatography device according to any of the preceding claims for generating very small sample values having a volume in a range from 1 nl to 20 μl, preferably having a volume in a range from 1 to 300 nL, most preferably having a volume in a range from 1 to 50 nL.

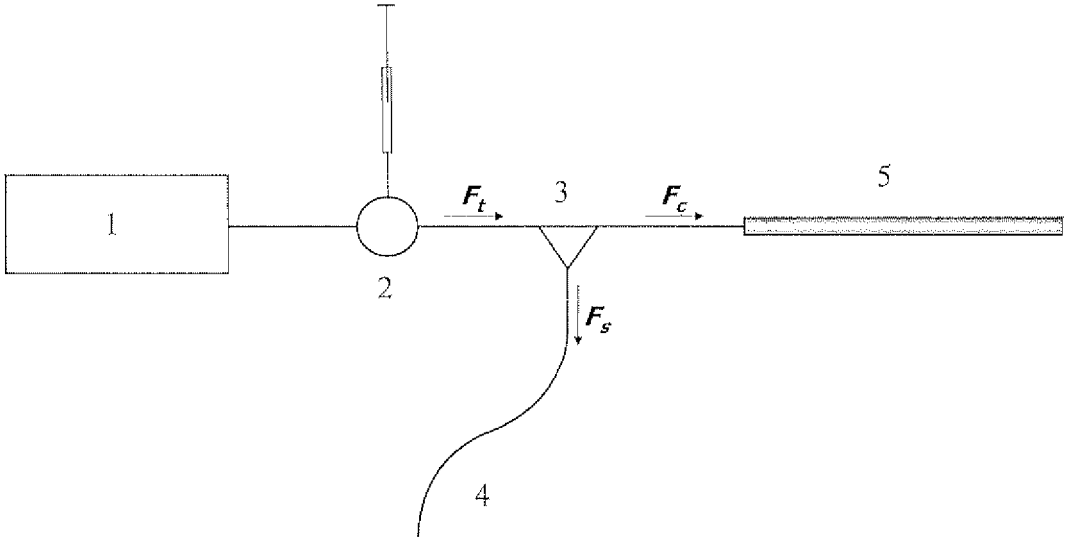
1
2
3
4
5
F_t
F_c
F_s

Fig. 1

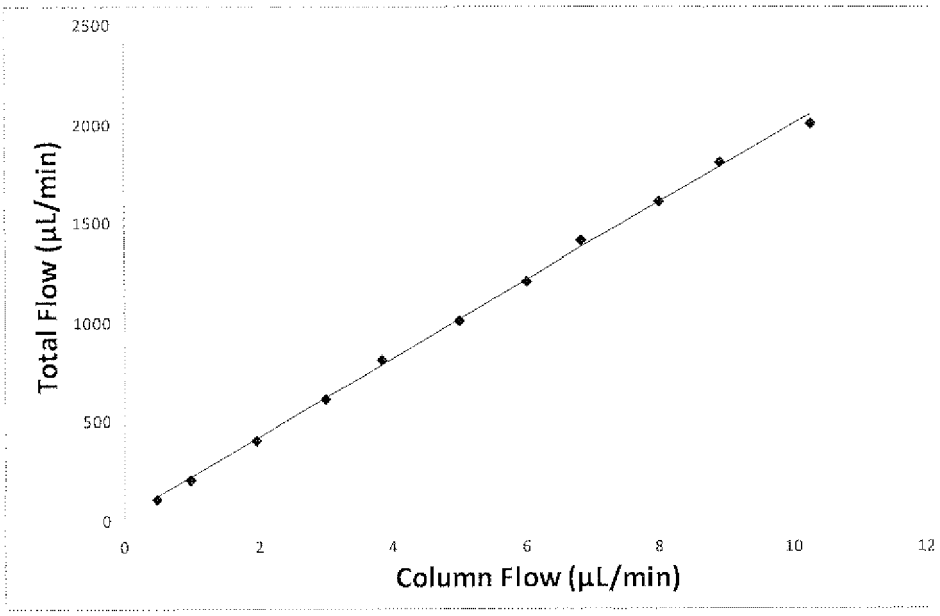
Total Flow (µL/min)
Column Flow (µL/min)
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12

Fig. 2

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 12 15 4228

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X A	EP 0 495 255 A1 (CHERVET JEAN PIERRE [NL] LC PACKINGS NEDERLAND BV [NL]) 22 July 1992 (1992-07-22) * page 2, lines 36-41; figure 2A * * page 3, lines 51-54 * * page 4, lines 5-8 * * page 4, lines 54-57 *	1,3,5-14 2,4	INV. G01N30/32 G01N30/20
X	TAKEUCHI T ET AL: "Split flow and bypass flow systems for monolithic capillary columns in liquid chromatography", JOURNAL OF CHROMATOGRAPHY, ELSEVIER SCIENCE PUBLISHERS B.V, NL, vol. 1021, no. 1-2, 22 December 2003 (2003-12-22), pages 55-59, XP004471337, ISSN: 0021-9673, DOI: 10.1016/J.CHROMA.2003.08.087 * page 56; figure 1A *	1,2	
X	EP 0 329 290 A2 (HEWLETT PACKARD CO [US]) 23 August 1989 (1989-08-23) * page 5, lines 9-47; figure 4 *	1,2	TECHNICAL FIELDS SEARCHED (IPC) G01N
X	DJORDJEVIC,N. M. AND HOUDIERE,F.: "Simultaneous programming of two parameters in micro- and capillary-HPLC", REVIEWS IN ANALYTICAL CHEMISTRY, vol. 17, no. 4, 1998, pages 207-221, XP002677638, Berlin Germany ISSN: 0793-0135 * page 210 - page 211; figure 1 *	4	
A	FR 2 192 853 A1 (PERKIN ELMER CORP [US]) 15 February 1974 (1974-02-15) * the whole document *	1-14	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	13 June 2012	Müller, Thomas

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

1

EPO FORM 1503 03.82 (P04C01)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 12 15 4228

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

13-06-2012

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0495255 A1	22-07-1992	AT 139625 T	15-07-1996
		DE 69120386 D1	25-07-1996
		DE 69120386 T2	31-10-1996
		EP 0495255 A1	22-07-1992
EP 0329290 A2	23-08-1989	DE 68918608 D1	10-11-1994
		DE 68918608 T2	09-02-1995
		EP 0329290 A2	23-08-1989
		JP 1269053 A	26-10-1989
		US 4845985 A	11-07-1989
FR 2192853 A1	15-02-1974	AU 5801273 A	16-01-1975
		CA 1004999 A1	08-02-1977
		CH 588073 A5	31-05-1977
		DE 2334624 A1	24-01-1974
		FR 2192853 A1	15-02-1974
		GB 1434987 A	12-05-1976
		IT 1011010 B	20-01-1977
		JP 49054095 A	25-05-1974
		US 3798973 A	26-03-1974

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 6289914 B1 **[0005]**
- US 7465382 B2 **[0006]**
- US 20090165873 A1 **[0007]**
- US 20070283746 A1 **[0008]**