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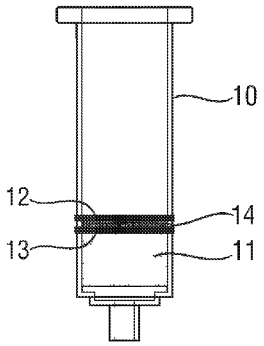
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(54) Title: MODIFIED CARTRIDGE WITH ADSORBENT POLYMER FOR SOLID-PHASE EXTRACTION (SPE)

Fig. 3



(57) Abstract: A solid phase extraction (SPE) cartridge for use in a fluorimetric assay comprises an elongate container (10) of a material permeable to fluorescent radiation, having an open inlet end and an open outlet end. For assay of a compound of interest, a solid adsorbent stationary phase (11) is provided in the container to adsorb the target compound from a mobile phase. To reduce background fluorescence that may interfere with the assay, at least one region (12,13) that is is at leastpartially impermeable to fluorescent radiation, for example by absorbence, scattering or refracting, is provided in or on the container wall, suitably on the outlet side and/or the inlet side of the anticipated location of a band or layer (14) of a compound of interest adsorbed in the stationary phase during an SPE procedure.

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MODIFIED CARTRIDGE WITH ADSORBENT POLYMER FOR SOLID-PHASE EXTRACTION (SPE)

This invention concerns an SPE (solid phase extraction)adsorbent cartridge which has been modified by providing masking regions that reduce background fluorescence.

Background of the invention

A wide variety of human foods and animal feeds, including edible nuts, oilseeds, cereal grains, and forages and products derived from them are susceptible to contamination by mycotoxins, which are toxic metabolic by-products of fungi which can occur on food and feed crops both before and after harvest. Among the most significant are the aflatoxins, and ochratoxins. Direct determination of mycotoxin level is an important aspect of quality control in foods and feeds.

Such measurements have conventionally been carried out by the use of high performance liquid chromatography (HPLC). However in those cases where HPLC equipment is not available or appropriate, determination by thin layer chromatography (TLC) is also possible. Commercial scanners are available for mycotoxin determination after TLC separation, using mercury lamps with an emission wavelength of 366 nm as a light source to stimulate fluorescence, which is detected and quantified by photo-multipliers. For quantitative testing there are also radioimmunoassay techniques and immunochemically-based techniques such as enzyme-linked immunosorbent assay (ELISA) methods.

Before a solution obtained by extraction from a foodstuff sample is subjected to quantitative measurement, using HPLC for example, the solution may be subjected to a 'clean-up' procedure using solid phase extraction to remove compounds that may interfere with the mycotoxin evaluation.

Qualitative detection of mycotoxins can be carried out using small chromatographic columns (so-called 'minicolumns') in which the mycotoxins are immobilised as a layer within a mineral adsorbent in the mini-columns. The minicolumns are viewed under ultraviolet light to cause the immobilised mycotoxin to fluoresce. Various minicolumn methods have been adopted as official tests of the AOAC International (Association of Official Analytical Communities) to test for the presence of mycotoxins.

For the quantitative assay of mycotoxins, WO 2006/123189 describes fluorometric apparatus which assesses not only mycotoxin samples immobilised in layers in minicolumns, but also in molecularly imprinted polymers and non-molecularly imprinted (blank) polymers provided as absorbents for mycotoxins in solid phase extraction (SPE) cartridges.

A typical commercially available (eg from Supelco of Poole, UK) SPE adsorbent cartridge is shown in cross-section in Fig. 1. A tubular, ie syringe-shaped, container 1 has a spout 2 at its outlet end and support flanges 3 at its inlet end. The container is made from a transparent plastics material that suitably has low inherent fluorescence, and which in more expensive grades is ideally non-fluorescent, under the conditions which stimulate fluorescence of mycotoxins. A porous frit 4, preferably of non-fluorescent material such as PTFE, is placed at the base of the container to prevent loss of solids through the outlet spout 2. A polymer that selectively adsorbs a mycotoxin is loaded in powder form into the cartridge to form a polymer body 5. A further retaining frit 6 may be placed against the upper surface of the polymer layer 5. Alternatively a cylindrical monolith of porous polymer is placed on top of the frit 4.

In use of the SPE unit, a liquid extract from a material potentially containing a mycotoxin is delivered as a mobile phase into the inlet end of the container and allowed to drain (naturally or under suction) through the absorbent layer 5, exiting from the outlet end via spout 2. Any mycotoxin in the sample is absorbed by the polymer and forms a layer or band in the upper region of the absorbent polymer body, typically just below the frit 6, if present.

Polymers that selectively adsorb aflatoxins and ochratoxins, and the use of the polymers in SPE cartridges, are disclosed in WO 2008/096179 and WO 2008/125887 respectively.

The SPE cartridges with adsorbed mycotoxin layer may be used for quantitative analysis of the adsorbed mycotoxin by inserting the cartridge into an analytical fluorimeter, for example, as described in WO 2006/123189, the entire disclosure of which is incorporated herein by reference. When measuring the fluorescent output from the adsorbed mycotoxin, the user must take account of background fluorescence generated by the material of the cartridge and the adsorbent polymer.

To reduce background fluorescence during the analysis, in this invention an masking region is provided on the cartridge, to shield the polymer and cartridge below, and/or optionally above, the position where the fluorescent band of adsorbed mycotoxin will appear.

Summary of the invention

Accordingly the present invention provides a cartridge for solid phase extraction comprising an elongate container whose walls are made of a material permeable to fluorescent radiation and having an open inlet end and an open outlet end, characterised in that one or more masking regions are provided in or on the container wall.

For use in a SPE procedure, a solid adsorbent stationary phase is provided within the container. In use of the cartridge in a fluorimetric assay, a mobile phase containing a compound of interest that is fluorescent (or can form fluorescent reaction products) is loaded through the open inlet end onto the stationary phase, passes through the stationary phase leaving a band or layer of the compound of interest adsorbed in the stationary phase and is discharged from the open outlet end.

The function of the masking region(s) is to diminish the amount of background fluorescence, that ma y be generated by the solid phase or the material of the cartridge or by other materials present within the cartridge after the SPE procedure, that reaches the sensor of the fluorimeter and obscures the signal from the adsorbed bad of the compound of interest. Suitably the masking region(s) absorb, refract or scatter fluorescent radiation that provides an unwanted background signal.

For convenience the invention is described below in terms of syringe-shaped cartridges containing polymeric SPE materials intended as adsorbents for mycotoxins, as described above with reference to Fig.1 . However it will be appreciated that the concepts of this invention may be applied to any cartridge or cuvette systems intended for use in fluorimetry, which are not necessarily of circular cross-section, and to alternative systems such as the minicolumns mentioned above or a rod or flat surface coated with adsorbent, or using non-polymeric solid phases such as the mineral adsorbents often used with minicolumns. Additional arrangements involve the application of the adsorbent onto the inner surface of a

column or onto the inner surface of a cuvette (see for example then system used by Piletsky *et al* in Biosensors and Bioelectronics, Volume 16, Issues 9-12, December 2001 , pp 701-707).

Also some compounds of interest, such as the above-mentioned aflatoxins and ochratoxins, are naturally fluorescent, whereas others require the addition of a derivatising agent to the mobile phase or to the stationary phase in order to produce a fluorescent derivative.

The cartridges of the invention are suitable for a very broad range of applications, wherever there is a need for a simple, accurate, inexpensive and rapid means of measuring a compound of interest, as well as the mycotoxins specifically mentioned. Other potential applications within the food sector include the measurement of pesticide and veterinary residues, algal toxins, illicit dyes (e.g. Sudan I), and indicators of food quality. Outside of the food sector, areas where the cartridges can potentially be used include the control of environmental pollutants, drug abuse and counterfeit drugs. Applications are also anticipated in the forensic and healthcare (point of care) sectors.

Brief description of the accompanying drawings

Figure 1 shows a conventional SPE cartridge in cross-section;

Figure 2 shows a modified cartridge of this invention;

Figure 3 shows a 1st modified cartridge of this invention;

Figure 4 shows a 2nd modified cartridge of this invention;

Figure 5 is a graphical display showing the effect of the modified cartridge of Fig. 3 in reducing background fluorescence;

Figure 6 is a graphical display showing the effect of the modified cartridge of Fig. 4 in reducing background fluorescence.

Detailed description of the invention

The present invention is concerned with a cartridge for solid phase extraction for use in a fluorimetric assay. The cartridge comprises an elongate container of a material permeable to fluorescent radiation, having an open inlet end and an open outlet end. For assay of a compound of interest,a solid adsorbent stationary phase is provided in the container to adsorb the target compound. The characteristic feature of the

invention is that at least one region that is is at least partially impermeable to fluorescent radiation, for example by absorbence, scattering or refracting, is provided in or on the container wall, suitably on the outlet side and/or the inlet side of the anticipated location of a band or layer of a compound of interest adsorbed in the stationary phase during a SPE procedure.

In a preferred embodiment the stationary phase is a packing of an adsorbent polymer in powder form, or a shaped monolith of an adsorbent polymer. Typically the stationary phase is positioned against a porous frit mounted in the outlet end of the cartridge. Also a porous frit is positioned against the stationary phase at its surface remote from the outlet end. Molecularly imprinted polymers and non-molecularly imprinted (blank) polymers may be used as adsorbent as described in WO 2006/123189. Non-molecularly imprinted polymers that selectively adsorb aflatoxins and ochratoxins, are disclosed in WO 2008/096179 and WO 2008/125887 respectively.

Suitably the impermeable region is a band that is at least partially opaque to fluorescent radiation, and which extends around the container on the outlet side of the anticipated position of the band or layer of the compound of interest adsorbed in the stationary phase. The at least partially opaque band may be a relatively narrow band or may extend around the container so as to cover the stationary phase from the base of the container to a level below the anticipated position of the band or layer of the compound of interest adsorbed in the stationary phase. In terms of an empty cartridge, the at least partially opaque band extends from the outlet end part way towards the inlet end

Additionally or alternatively an at least partially opaque band extends around the container on the inlet side of the anticipated position of the band or layer of the compound of interest adsorbed in the stationary phase

The opaque band may be formed from adhesive tape applied to the container, such as masking tape or electric insulation tape, preferably black tape, after loading the polymer. Alternatively, the tape can be applied before loading the polymer, if the upper level of the polymer can be accurately predicted.

In a commercial process, where the adsorbent polymer is loaded in accurately controlled conditions, a container may be provided with an at least partially opaque areas formed during manufacture, for example, by coating or spraying with an opaquing material containing a pigment or dye, preferably black. Alternatively in a commercial cartridge forming process, the opaque band may be formed from pigment or dye incorporated in the material forming the container

This technique for reducing background fluorescence is applicable to both conventional SPE cartridges and the previously mentioned mini-columns with mineral adsorbents, or any analytical system where a fluorescent band of adsorbed compounds is created in an adsorbent material supported in a cartridge or cuvette.

The invention is illustrated in the accompanying drawings. Fig. 2 shows a typical SPE adsorbent cartridge in which a transparent syringe-shaped container 10 having a porous frit at its base is loaded with a macroporous polymer which is selectively adsorbent for a compound of interest. The polymer is typically in powder form and the polymer layer is topped with another porous frit.

After carrying out an SPE procedure with a solution containing a fluorescent compound of interest, a layer of adsorbed compound forms below the upper frit, typically in a region near the upper surface of the polymer 11, shown as band 14 in Fig. 3. To reduce background fluorescence during a fluorimetry assay using the cartridge, a strip of black adhesive tape is applied to the cartridge as a band 13 just below the layer of adsorbed compound preferably also a band 12 just above the upper frit. The upper frit provides a marker for the position of the upper band 12. If the compound layer 14 is not visible to the naked eye then the lower band 13 is applied below the upper surface of the polymer layer, with a sufficient leeway to avoid obscuring the layer 14.

If the position of the upper surface of the polymer layer is predictable ie when standardized amounts of polymer are loaded, then the bands 12 and 13 can be positioned prior to the SPE procedure within tolerances which will result in the compound layer 14 being formed between the bands 12 and 13. Alternatively, as mentioned above, when the polymer is loaded in a standardized procedure for use

with specific compounds, empty cartridges 10 can be provided with preformed opaque regions by selective coating.

In the alternative configuration shown in Fig. 4, a black adhesive tape 15 is applied to a cartridge 10 so as to cover a greater portion of the polymer layer 11, typically extending from the base of the cartridge to a height which allows sufficient clearance so that the compound layer 14 is not obscured. This configuration is conveniently reproduced as a pre-formed unit by dip-coating an empty cartridge in a coating solution containing a pigment or dye to form a coating which adsorbs, refracts or scatters background fluorescence.

The invention is further illustrated by the following Examples.

Example 1

Preparation of Polymer and SPE Cartridge

A polymerisation mixture was prepared by stirring the functional monomer methylene bisacrylamide (MBAA), 5g; a cross-linker ethylene glycol dimethacrylate (EGDMA), 2Og, a porogen N,N-dimethylformamide (DMF), 25g; and an initiator 1,1-azobis(cyclohexanecarbonitrile), 500mg. The polymerisation mixture was illuminated for 20 min using a Honle 100UV lamp (intensity 0.157 W/cm^2) (Honle UV, UK) followed by thermo-annealing in an oil bath at 80 ^{0}C for 12 hours. Washing with methanol removed the solvent (DMF) and the resulting polymer was a macroporous material. The resultant bulk polymers were ground and wet-sieved in methanol. The fraction with particle size in the range from 25 to 63 μm was collected and dried.

After preparation, 75mg of polymer were added to empty 1 ml non-fluorescent plastic cartridges (Supelco UK) fitted with non-fluorescent Teflon (PTFE) frits (Supelco UK), The resultant SPE adsorbent cartridges have a structure as illustrated in Fig. 2.

Example 2

Immobilisation of AFB1 in SPE Cartridge

Cartridges prepared as in Example 1 were pre-conditioned by addition of 2 ml water (e.g. HPLC grade water). Solutions of aflatoxin B1 (AFB1) in 4 ml of methanol (concentration of methanol varied from 80% to 20%, concentrations of AFB1 varying from 10 to 200 ng) were added to the cartridge to load the aflatoxins onto the layer of

MBAA polymer. The cartridge was then washed to remove interfering compounds using 1 ml of 20% methanol (80:20, water:methanol). The presence of a layer of the aflatoxins immobilised in the layer of MBAA polymer was shown by observing the fluorescence generated by exposure to UV light, by utilising a sensor device as disclosed in WO 2006/1 20381 .

A sharp fluorescent band was observed directly under the top frit of the SPE cartridge. The sensitivity of detection of AFB1 on the MBAA-based polymer using a Toximet T1 sensor device (Toximet Ltd, UK), as disclosed in WO 2006/120381 ,was very good, especially when adsorbed from 20% methanol.

Example 3

1st Modification of the optical characteristics of the polymer cartridge

The intensity of the background signal when measuring the fluorescence of the AFB1 band in Example 2 was successfully reduced by modifying the optical characteristics of the cartridges.

Thin strips of black masking tape were placed around the circumference of the cartridge immediately above and below the band of immobilised mycotoxin as shown in Fig. 3. When the fluorescence of the mycotoxin was measured using a Toximet T1 sensor device, the background fluorescence of the MBAA-based polymer, produced and loaded in a cartridge as in Example 1, and used to immobilise AFB1 as in Example 2, was significantly reduced as shown in Fig. 5

Fig. 5 shows the high quality signal generated by aflatoxin B_1, where the background radiation emitted immediately above and below the toxin band has been masked as shown in Fig. 3. In the graphical display provided by the Toximet T1 instrument, line 31 shows the signal generated by a sample containing 20 mg AFB1 and line 32 the signal generated by a sample containing 10 mg AFB1 . The reduced background signal is shown by line 33 - the background has been reduced from about 140,000 to below 40,000 units.

Example 4

2nd Modification of the optical characteristics of the polymer cartridge

The intensity of the fluorescent background was also significantly reduced by the addition of a broad circumferential band of masking tape to a cartridge of the type in Fig 2, the blacked out region extending from the base of the cartridge to a position below the immobilised aflatoxin band, as shown in Fig. 4. This significantly reduced the background fluorescence, even on the part which was not covered with tape.

When the fluorescence of the mycotoxin was measured using a Toximet T1 sensor device, Fig. 6 shows that the background fluorescence was reduced from 120, 000 units (line 41) to 20,000 units (line 43), and that 10 ng of aflatoxin B_1 gave a well-defined signal equivalent to 70,000 units (line 42).

CLAIMS

1. A cartridge for solid phase extraction comprising an elongate container whose walls are made of a material permeable to fluorescent radiation and having an open inlet end and an open outlet end, characterised in that one or more masking regions are provided in or on the container wall.

2 A cartridge according to claim 1 in which the masking region is formed from an adhesive tape applied to the container.

3. A cartridge according to claim 1 in which the masking region is formed from pigment or dye in a coating layer applied to the container walls.

4. A cartridge according to claim 1 in which the masking region is formed from pigment or dye incorporated in the material forming the container walls.

5. A cartridge according to any preceding claim in which the masking region extends from the outlet end part of the way towards the inlet end.

6. A cartridge according to any preceding claim further comprising a solid adsorbent stationary phase within the container.

7. A cartridge according to claim 6 in which the stationary phase is a packing of an adsorbent polymer in powder form.

8. A cartridge according to claim 6 in which the stationary phase is a shaped monolith of an adsorbent polymer.

9. A cartridge according to claim 6, 7 or 8 in which the stationary phase is positioned against a porous frit mounted in the outlet end.

10. A cartridge according to any one of claims 6 to 9 in which a porous frit is positioned against the stationary phase at its surface remote from the outlet end.

11. A cartridge according to any one of claims 6 to 10 in which the masking region covers a region of the container wall excluding the anticipated position intermediate the outlet and the inlet end of a band or layer of the compound of interest adsorbed in the stationary phase after use of the cartridge for a solid phase extraction procedure.

12. A cartridge according to claim 11 in which a masking region extends around the container on the outlet side of the anticipated position of the band or layer of the compound of interest adsorbed in the stationary phase.

13. A cartridge according to claim 11 in which a masking region extends around the container on the inlet side of the anticipated position of the band or layer of the compound of interest adsorbed in the stationary phase.

14. A cartridge according to claim 11 in which a masking region extends around the container on the inlet side and on the outlet side of the anticipated position of the band or layer of the compound of interest adsorbed in the stationary phase.

15. A cartridge according to claim 11 in which a masking region extends around the container so as to cover the stationary phase except in the proximity of the anticipated position of the band or layer of the compound of interest adsorbed in the stationary phase.

16. A cartridge according to any preceding claim in which the masking region(s) at least partially absorb, refract or scatter background fluorescence.

Fig. 1

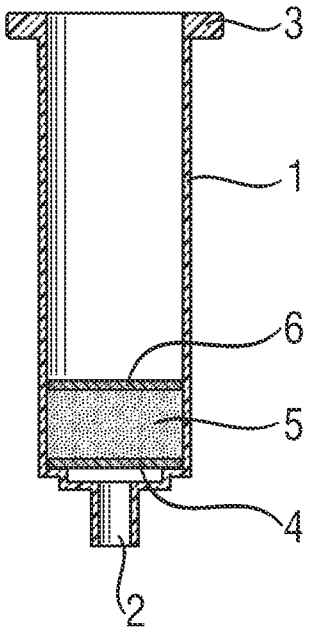


Fig. 2

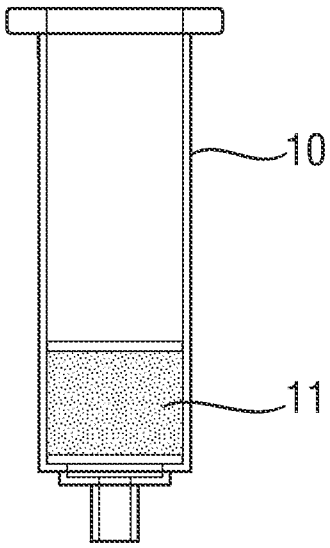


Fig. 3

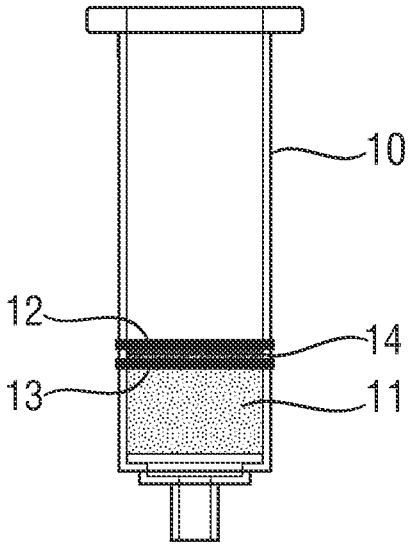


Fig. 4

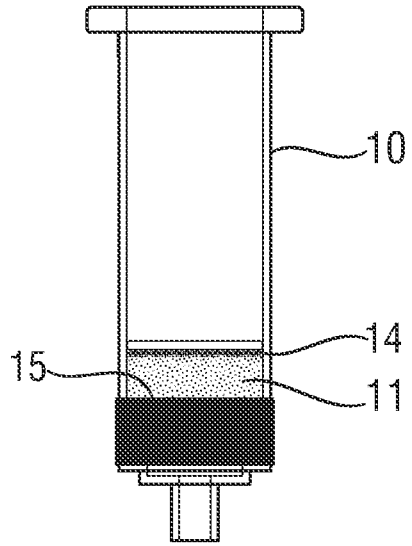


Fig. 5

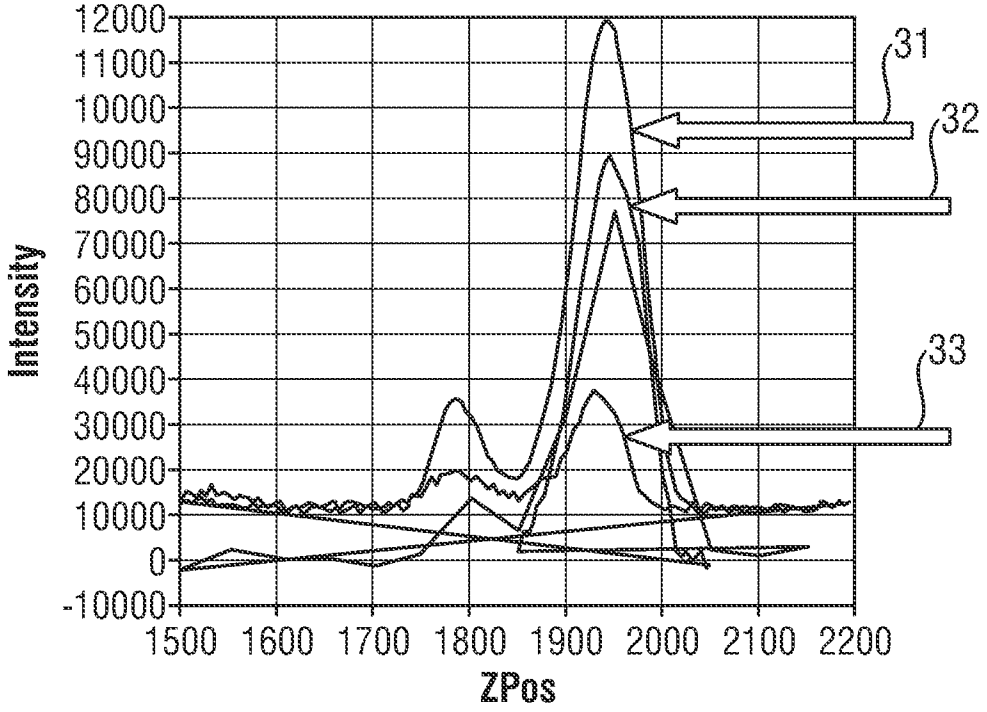
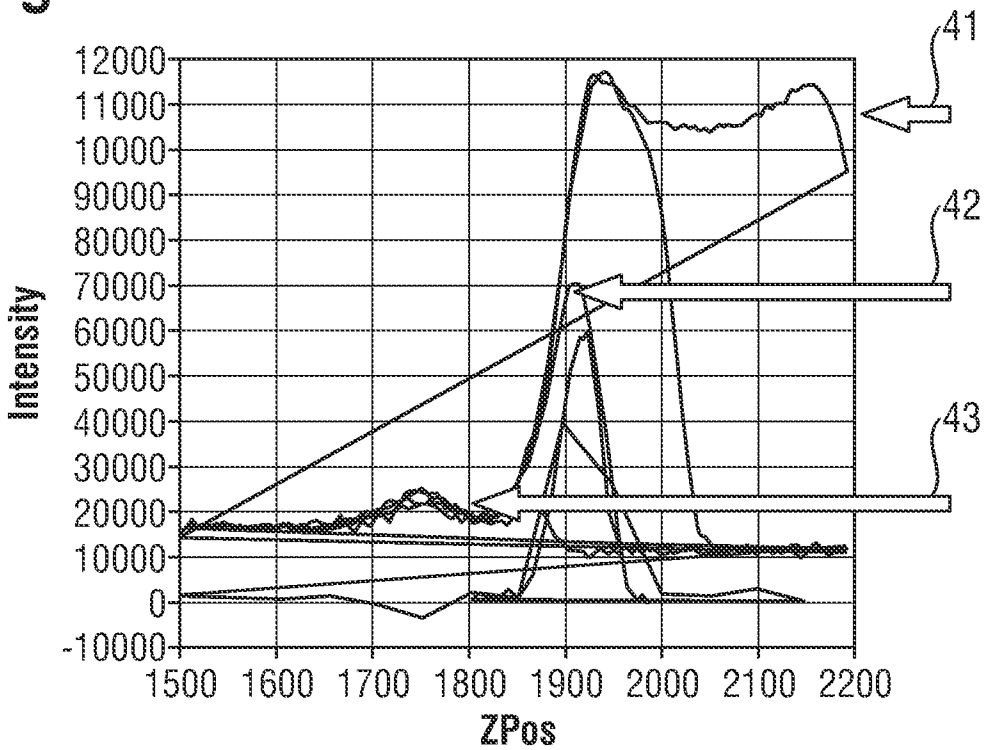

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Fig. 6


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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/050126

A. CLASSIRCATION OF SUBJECT MATTER
INV. G01N1/40

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)
GOIN

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 practical search terms used)

EPO-Internal , WPI Data, **COMPENDEX, INSPEC**

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to claim No
X	**WO 2006/123189 A (UNIV GREENWICH [GB]; COKER RAYMOND DOUGLAS; NAGLER MARTIN JESSE) 23 November 2006 (2006-11-23) abstract; figure 7 page 13, lines 4-10**	**1-16**
P,X	**WO 2008/125887 A (UNIV GREENWICH [GB]; PILETSKY SERGEY [GB]; PILETSKA OLENA [GB]; COKER) 23 October 2008 (2008-10-23) cited in the application abstract; figures** 1,2 **page** 7 , **line 32 - page 8 , line 11**	**1-16**

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[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
22 April 2009	**07/05/2009**

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P B 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel (+31-70) 340-2040 Fax (+31-70) 340-3016	Faderl , Ingo

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/050126

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2008/096179 A (UNIV GREENWICH [GB]; PILETSKA OLENA [GB]; COKER RAYMOND [GB]; PILETSKY) 14 August 2008 (2008-08-14) cited in the application abstract; figures 1,2 page 17; example 25	1,2,5-16
A	DE 42 08 732 A1 (ABION OHG [DE]) 30 September 1993 (1993-09-30) abstract; claims; figure Ia	1-16
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2009/050126

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