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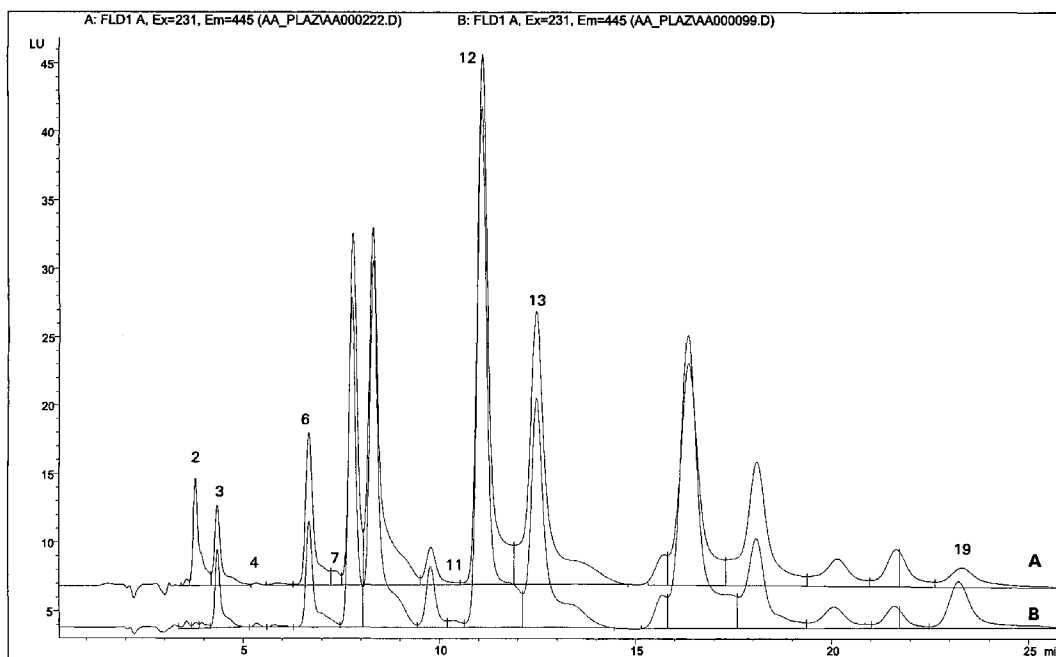
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(54) Title: EX VIVO CANCER DIAGNOSTIC METHOD



(57) Abstract: The present invention relates to an ex vivo diagnostic method for the detection of malignant tumorigenic development or cancer in a human individual, the method being based on the determination of qualitative and/or quantitative alterations of at least one compound, preferably of an amino acid or an amino acid related compound, present in a bodily fluid of a diseased individual as opposed to a healthy control.

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EX VIVO CANCER DIAGNOSTIC METHOD

FIELD OF THE INVENTION

The present invention is in the field of medical diagnostics and relates to an
5 *ex vivo* method of detection of a pathological condition, particularly of a
malignant tumor, in a human or non-human mammalian individual.

BACKGROUND OF THE INVENTION

10 In the fight against cancer early and reliable detection of tumorigenic
development and of the existence and/or distribution of cancer cells is of
crucial importance. Currently, early detection programs, including screening
and testing, are available for only a few of the most common cancers. For
instance, the "American Cancer Society Guidelines for the Early Detection of
15 Cancer, 2004" recommend screening for breast, cervical, colorectal,
endometrial, prostate, and lung cancers. Almost all existing cancer screening
tests are designed to detect just one specific form of cancer and cannot be
used to indicate the presence of other cancers. Further, at least some of
these tests are of low sensitivity even though fairly expensive.

20

It is known that various pathological conditions and diseases, and
particularly cancer development, are typically associated with or even
caused by defects in the immunological defense system. For instance,
compounds affecting the protein synthesis in the cells of the immune system
25 have significantly different functionality in healthy individuals as compared
to cancer patients.

BRIEF DESCRIPTION OF THE INVENTION

30 In the attempt to find a reliable, *ex vivo* diagnostic method for the detection
and/or confirmation of malignant tumorigenic development in an individual, it
has been found that blood cells, particularly white blood cells, obtained from

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cancer patients differ significantly over their counterparts obtained from healthy blood donors as regards their physiological status and/or metabolic activity. For instance, the cells of diseased patients differ from the ones of healthy donors *inter alia* in their ability to take up nutritional compounds
5 such as, e.g. amino acids, from their natural biological environment as well as from an artificial environment such as a suitable *ex vivo* nutrient medium. This effect was found to be even more pronounced in the presence of certain alkaloid derivatives *in vitro*, particularly in the presence of one or more alkaloid derivatives having a quaternary nitrogen atom such as the
10 ones referred to in WO 2004/082698, which effect has already been reported in the co-pending patent application WO 2006/053680 (Appl.No. PCT/EP2005/012083), the contents of which shall be entirely incorporated herein.

It has now been found, however, that such differences in the physiological
15 status and/or metabolic activity of blood cells and, as the case may be, also of body tissues between healthy and diseased individuals can be monitored even in the absence of any exposure of such cells to external agents such as the above-mentioned nutrient or alkaloid compounds, namely by detecting and evaluating differences in the composition of body liquids obtained from
20 healthy and diseased individuals.

Based on the exciting finding of this general principle a fully *ex vivo* diagnostic method for the determination of a pathological condition in an individual was established using the different cellular characteristics of body
25 liquids, e.g. of red and/or white blood cells of diseased patients, as compared to corresponding body liquids, e.g. to corresponding red and/or white blood cells of healthy donors, as a measure for the prediction of the presence of a malignancy, particularly of a malignant tumorigenic activity and most particularly for the detection of a cancer disease in an afflicted
30 individual.

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This *ex vivo* method is extremely patient-friendly as it does not require administration of any possibly hazardous agent to a patient solely for the purpose of cancer diagnostics.

5 DETAILED DESCRIPTION OF THE INVENTION

It was recognized that the concentration profiles of various compounds present in the blood plasma, in saliva or in sweat samples obtained from diseased and from healthy donors, differ significantly from each other and
10 thus allow for a prediction of the presence of malignancy, particularly of tumorigenic development or an established cancer in a mammal, i.e. in an animal or human individual.

The recognition of this principle led to set up an *ex vivo* diagnostic method
15 for the detection and/or confirmation of the presence of malignancy in a tested individual.

Additionally, it was found that the different physiological status and/or metabolic activity of blood cells is also detectable even without the addition
20 of a cell culture medium or of nutritional compounds.

The term "amino acid" as used herein relates to any known L- or D-amino acid known to occur in human or animal individuals, including all essential and non-essential amino acids and all other naturally occurring amino acids.
25

The term "amino acid related compound" as used herein mainly relates to modified amino acids, amino acid derivatives and metabolites, and amino acid-based compounds such as smaller peptides naturally occurring in the animal or human body.
30

The term "compound fraction" as used herein relates to an amino acid fraction, a fraction comprising an amino acid related compound or any other compound.

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The present invention therefore in its first aspect relates to a method of detecting a pathological condition, particularly the presence of a malignancy, more particularly of a malignant tumor or a cancer disease, in an individual, the method comprising:

- 5 - obtaining a sample of a bodily fluid from said individual;
- obtaining a cell-free liquid fraction of said sample;
- subjecting said cell-free fraction to analytical determination of at least one amino acid and/or amino acid related compound, preferably of a plurality of amino acids and/or amino acid related compounds, present in said
10 fraction; and
- evaluating qualitative and/or quantitative results of said analytical determination against corresponding values obtained from control samples derived from healthy blood donors,

wherein statistically significant qualitative and/or quantitative differences in
15 the values of said individual as compared to the controls indicates a pathological condition, particularly the presence of a malignancy, more particularly of a malignant tumor or of cancer cells or tissues.

In a further aspect the invention relates to such a method, wherein said
20 body liquid sample is selected from the group consisting of a whole blood sample, typically a heparinised whole blood sample, a saliva sample, and a sweat sample.

In a further aspect the invention relates to such a method, wherein said
25 method comprises separating said cell-free liquid fraction into single compound fractions by liquid chromatography, preferably by HPLC, and detecting at least one, preferably a plurality of compound fractions, present in said cell-free fraction, using a suitable detection system.

30 In a further aspect the invention relates to such a method, which comprises detecting in the control sample at least one compound fraction which is not

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detected, or only at a significantly lower concentration, in the sample obtained from a said individual.

This significantly lower concentration is typically in a range of equal or less than 20 %, preferably equal or less than 10%, of the corresponding values of a said compound fraction in a pooled control.

In a further aspect the invention relates to such a method, wherein under the analytical conditions referred to in Example 1 at least one of said compound fractions present in the control sample but not or at a significantly lower concentration in the sample of a said individual elutes at a retention time prior to aspartic acid.

While samples obtained from patients suffering from manifest cancers were typically void of said particular compound fraction eluting at a retention time prior to the corresponding retention time of aspartic acid, some samples obtained from early stage cancer patients exhibited very low concentrations of said amino acid or related compound, i.e. concentrations of equal or less than 20 %, mostly of equal or less than 10% and typically of less than 5% of the corresponding values of the control.

Subsequent analysis revealed that said compound fraction which may qualify as a "cancer marker compound fraction" elutes at the same retention time as glutathione.

25

In a further aspect the invention relates to such a method, wherein under the analytical conditions referred to in Example 1 one of said compound fractions present in the control sample but not or at a significantly lower concentration in the sample of said individual elutes at a retention time of about 2.5 min, e.g. at 2.57 min, and/or another one at about 3 min, e.g. at 3.07 min, and/or another one at about 3.7 min, e.g. at 3.79 min, while aspartic acid elutes at about 4.3 min, e.g. at 4.34 min.

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In a further aspect the invention relates to such a method which is suitable for early prognosis on the risk of cancer development in a said individual.

5 In a further aspect the invention relates to such a method, wherein the detection of a compound fraction present in the sample of a said individual at a concentration significantly lower than in a pooled control is indicative of an increased risk of developing cancer or of the presence of an early stage cancer in said individual.

10 In a further aspect the invention relates to such a method, wherein a failure to detect in the sample of a said individual a compound fraction present in a pooled control is indicative of the presence of a malignant tumor or of cancer cells or tissues in said individual.

15 In a further aspect the invention relates to such a method, wherein said compound fraction comprises a compound that elutes prior to aspartic acid under the analytical conditions referred to in Example 1.

20 In a further aspect the invention relates to such a method which comprises detecting at least one compound fraction present in the experimental sample and not in a pooled control sample.

25 In a further aspect the invention relates to such a method, wherein under the analytical conditions referred to in Example 1 at least one of said compound fractions present in the experimental sample and not in the control sample elutes at a retention time later than that of alanine.

30 In a further aspect the invention relates to such a method, wherein under the analytical conditions referred to in Example 1 one compound fraction present in the experimental sample and not in the control sample elutes at a retention time of about 50 min and/or another one compound fraction

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present in the experimental sample and not in the control sample elutes at a retention time of about 70 min.

In another aspect, the present invention relates to the above described method which further comprises quantitative evaluation of the concentration of the amino acid or amino acid related compound corresponding to said product peak appearing ahead of the aspartic acid peak, for early prognosis on the risk of cancer development in said individual. If preliminary results hold true, it can be inferred from this method that if in a said individual a concentration level of said amino acid or amino acid related compound is detected of less than 20 %, particularly of less than 10 %, of the concentration level of a pooled control such low concentration is indicative of an increased risk of developing cancer or of the presence of an early stage cancer in said individual.

15

In order that the invention described herein may be more fully understood, the following examples are set forth. They are for illustrative purposes only and shall not be construed as limiting this invention in any respect.

20 BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 represents a concentration profile of amino acids and amino acid related compounds determined by HPLC and fluorescence detection from a healthy volunteer (control sample); ordinate = light units, abscissa = time in minutes.

25

Table 1: Legend of Fig. 1

Peak Number	Compound	Area beneath Peak	Retention time (min)
1	n.d.	23.9022	3.067
2	n.d.	7.77134	3.241
3	n.d.	6.88435	3.561
4	n.d.	155.859	3.791
6	Asp	82.2382	4.348

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7	n.d.	16.5053	5.347
8	n.d.	11.7576	5.899
9	n.d.	130.657	6.693
10	n.d.	18.9597	7.122
11	Glu	264.717	7.819
12	Ser	393.692	8.331
13	n.d.	48.9981	9.806

n.d.: not determined

Fig. 2 represents a concentration profile of amino acids and amino acid related compounds determined by HPLC and fluorescence detection from a blood plasma sample of a colon cancer patient; ordinate = light units, abscissa = time in minutes.

Table 2: Legend of Fig. 2

Peak Number	Compound	Area beneath Peak	Retention time (min)
3	n.d.	7.37068	3.554
4	n.d.	3.06662	3.785
5	n.d.	7.89172	3.947
6	Asp	105.851	4.348
7	n.d.	6.73086	5.341
8	n.d.	6.42904	5.805
9	n.d.	173.474	6.693
11	Glu	531.5	7.766
12	Ser	679.619	8.317
13	n.d.	47.5149	9.773

n.d.: not determined

10

Fig. 3 represents an overlay of the concentration profiles of Fig. 1 and Fig. 2; A = Fig. 1, B = Fig. 2; ordinate = light units, abscissa = time in minutes. A detailed legend for the concentration profiles A and B is found in Table 1 and Table 2, respectively.

15

From Figures 1 and 2 and especially from overlay in Fig. 3 it can easily be derived that two product peaks (peaks 1 and 4) appear in the control sample

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of a healthy volunteer, whereas the same product peaks are absent from the sample of a cancer patient. Apparently, metabolic products present in the blood of the healthy control have been utilized or further decomposed by the organism of the cancer patient and thus do not show up in the LC or HPLC chromatograms of the experimental samples. Based on this recognition the present inventor has developed an *ex vivo* cancer diagnostic method as herein described and claimed.

Fig. 4 represents a profile of amino acids and amino acid related compounds determined by HPLC and fluorescence detection of blood plasma from a healthy volunteer (control sample); ordinate = light units, abscissa = time in minutes.

Table 3: Legend of Fig. 4

Peak Number	Compound	Retention time (min)
1	CA	3.563
2	n.d.	3.785
3	Asp	4.344
5	n.d.	5.891
6	Asn	6.679
7	n.d.	7.329
8	Glu	7.801
9	Ser	8.312
10	n.d.	9.784
12	Gln	11.097
13	His	12.483
14	n.d.	15.741
15	Gly	16.371
16	Thr	18.099
17	PEA	20.150
18	n.d.	21.650
19	Arg	23.311
20	Tau	31.755
22	Ala	35.803

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23	Homotau	37.318
25	GABA	72.525
26	Tyr	77.851

n.d.: not determined

Fig. 5 represents a profile of amino acids and amino acid related compounds determined by HPLC and fluorescence detection of blood plasma of a stomach cancer patient (experimental sample); ordinate = light units, abscissa = time in minutes.

Table 4: Legend of Fig. 5

Peak Number	Compound	Retention time (min)
2	n.d.	3.794
3	Asp	4.345
4	n.d.	5.353
5	n.d.	5.801
6	Asn	6.682
8	Glu	7.773
9	Ser	8.319
10	n.d.	9.775
11	n.d.	10.356
12	Gln	11.086
13	His	12.485
14	n.d.	15.696
15	Gly	16.355
16	Thr	18.076
17	PEA	20.063
18	n.d.	21.603
19	Arg	23.231
20	Tau	31.755
21	bAla	33.986
22	Ala	35.811
23	Homotau	37.259
24	n.d.	38.578

25	GABA	72.491
26	Tyr	77.794

n.d.: not determined

Fig. 6 represents an overlay of the profiles of Fig. 4 and Fig. 5; A = Fig. 4, B = Fig. 5; ordinate = light units, abscissa = time in minutes. A detailed legend for the profiles A and B is found in Table 3 and Table 4, respectively.

Fig. 7 represents an enlarged and stretched view of a part of Fig.6.

From Figures 4 and 5 and especially from the overlay in Fig. 6 and Fig. 7 it can be derived that one product peak appears in the control sample of a healthy volunteer but not in the sample of a cancer patient (peak 2). More specifically, under the conditions applied herein an amino acid or amino acid related compound elutes under the chosen HPLC conditions shortly before aspartic acid (peak 3), which compound appears to be very much decreased in its concentration or is entirely absent (i.e. not detectable by this HPLC method) in the sample of the stomach cancer patient (experimental sample).

Moreover, a comparison of the chromatogram of the stomach cancer patient with the one of the healthy control represents that the peaks occurring at 5.35 min (peak 4), 10.35 min (peak 11), 23.23 min (peak 19; arginine), 33.99 min (peak 21; β -alanine) and 38.58 min (peak 24) are very low or entirely missing in the plot of the control while they are rather prominent in the plot of the cancer patient.

25

Also, it can be seen that in the chromatogram of the healthy control (Fig.4) peaks show up at a retention time of approximately 3.8 min (peak 4) and 7.3 min (peak 7) which are absent from the chromatogram of the cancer patient (Fig.5). The compound eluting at approx. 3.8 min under the given HPLC conditions is believed to be glutathione, as subsequent runs under

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identical HPLC conditions using *inter alia* glutathione as an amino acid marker showed that glutathione elutes at a retention time of approx 3.8.

5 Additionally, it was found that the concentration of asparagine (peak 6), glutamine (peak 12) and histidine (peak 13) tends to be lower in the sample of cancer patients as compared to healthy controls.

Example 1: Determination of metabolite profiles in blood samples of cancer patients and healthy donors

10

Method:

5ml venous blood were taken from a lymphoma or prostate cancer patient and transferred to a heparinized glass vial. 1ml thereof was transferred into an Eppendorf microcentrifuge glass container and centrifuged for 15 min at 1500 g.

20 The such obtained plasma was deproteinized by admixing an equal volume of 1M perchloric acid (HClO₄) and centrifuging the mixture for 20 minutes at 12000 g. As a control, pooled venous blood from healthy donors was used and was subjected to the same procedure described above .

As an internal standard, homotaurine (Sigma Aldrich) was used. The protein-free extracts obtained by this procedure were stored at -20°C and kept for subsequent analysis.

25

Various compounds including a number of amino acids were determined qualitatively and quantitatively by HPLC (Agilent 1100), applying isocratic elution from a Diasorb 130 C₁₈ T, 6 micrometer, 3x250 nm column (Elsiko, Russia); mobile phase: 0.1 M sodium acetate buffer, ph 5.71 – acetonitril:water (1:1) 65:35; flow rate 0.6 ml/min, 30°C. As a standard, a commercially available amino acid mixture (L-Amino Acid Kit, Sigma-Aldrich) was applied. The statistical evaluation was done using the software Statistica 6.0.

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Results:**Table 5: Presence/absence of low molecular weight compounds in blood plasma of lymphoma and prostate cancer patients**

Retention time (min)	Compound	Experimental samples	Control (healthy donors)
2.57	n.d.	-	+
50	n.d.	+	-
70	n.d.	+	-

5 n.d. = not determined

Conclusion:

The concentration values of some low molecular weight compounds, more specifically of some amino acids or amino acid related compounds determined from the plasma fraction of said blood samples exhibit significant differences in samples from healthy probands as compared to those from diseased patients, e.g. cancer patients. More specifically, whereas a product peak appears at a retention time of 2.57 min in the pooled plasma samples of the control group of healthy volunteers and not in those of the experimental group, two other product peaks appear at retention times of 57 and 70 min in the experimental samples (Table 5).

It seems that the peaks may not be attributed to any of the twenty natural amino acids usually present in a human body, but instead are assumed to be amino acid derivatives or related amino compounds, e.g. polar metabolic reaction products bearing at least one amino group. The concentration profile, i.e. the absence or presence of compounds at certain retention times under the given analytical conditions, obtained from the experimental samples as compared to the concentration profile obtained from the controls gives statistically relevant information as to the presence of malignancy in an individual.

Example 2: Determination of concentration profiles of selected amino acids and amino acid related compounds in blood samples of cancer patients and healthy donors

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Blood samples of a colon cancer patient and of a healthy donor have been prepared according to Example 1. The plasma fractions obtained therefrom have been analysed according to Example 1.

5 Results:

Table 6: Concentration profiles of selected amino acids and amino acid related compounds in blood plasma of a colon cancer patient and a healthy donor.

Peak number	Compound	Area beneath peak	
		Experimental sample	Control (healthy donor)
1	n.d.	-	23.9
4	n.d.	-	155.86
6	Asp	105.9	82.2
9	n.d.	173.5	130.7
11	Glu	531.5	264.7
12	Ser	679.6	393.7

10 n.d. = not determined

Conclusion:

The concentration values of selected amino acids and amino acid related compounds determined from the plasma fraction of said blood samples exhibit significant differences in the control samples of the healthy proband as compared to the experimental samples of the diseased patient, i.e. cancer patient. Whereas some of the determined compounds have been found to have reduced levels in the experimental plasma sample as compared to the sample of the healthy volunteer, other compounds have been found to have increased levels, as can be seen in Table 6 and Figures 1 to 3. More specifically, whereas two peaks appear at retention times of 3.07 min (peak 1) and 3.79 min (peak 4) in the control samples, these product peaks are absent from the experimental samples as can be seen in Figures 1 to 3. The concentration profile and/or the absence of distinct product peaks from the experimental samples gives statistically relevant information as to the presence of malignancy in the tested individual.

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Example 3: Determination of profiles of amino acids and amino acid related compounds in blood samples of cancer patients and healthy donors

5 Starting from the premise that tumor growth is accompanied by certain shifts in the formation of pools of amino acids and/or amino acid related compounds it was undertaken to monitor the changes in the content of amino acids and amino acid related compounds for cancer detection. 5ml venous blood were taken from 29 patients with different types of
10 cancer (stomach, rectal, lung, breast, bladder, prostate, and leukemia). Also 3 patients with benign processes and 10 healthy donors were included. All patients were investigated before treatment. Heparinized blood samples of patients were incubated for 2.5 h at 37°C in Eppendorf tubes without and with 100 µl of Ukrain® , diluted to 1:5, 1:10, 1:100 or 1:1000, followed by
15 a centrifugation step for 15 min at 1500 g in an Eppendorf microcentrifuge. The such obtained plasma was deproteinized by admixing an equal volume of 1M perchloric acid (HClO₄). Homotaurine (Sigma) was used as an internal standard. The protein-free extracts obtained by this procedure were stored at -20°C and kept for subsequent analysis according to Example 1.

20

Results:

In the plasma samples of cancer patients (see Fig. 5) as compared with the plasma samples of healthy controls (see Fig. 4) it was observed that in the samples that were not subjected to UKRAIN® treatment the plasma levels of
25 histidine, asparagine and glutamine (peaks 6, 12 and 13, respectively) tended to be lower in cancer patients than in healthy subjects, while the plasma levels of arginine and β-alanine (peaks 19 and 21, respectively) tended to be higher in cancer patients than in the healthy controls. A few unidentified peaks were found under the applied HPLC conditions which
30 occurred only in cancer patients and not in healthy controls, while one peak occurred in healthy subjects and not, or at very low levels only, in cancer patients, which latter peak (peak 2) typically showed up shortly before the aspartic acid peak as can be seen in Tables 3 and 4 and in Figures 4 to 7. Subsequent tests using additional marker compounds have elucidated (data
35 not shown herein) that under the same HPLC conditions glutathione elutes at identical or nearly identical retention times thus indicating that the

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compound present in the healthy control and absent or reduced in the cancer patients may very likely be glutathione.

Further useful information can be derived from the plots in Figures 1 to 7.

5

Since the experiments showed that the area beneath said "cancer marker" peak ahead of the aspartic acid peak may vary among the healthy controls such finding raises the question whether quantitative evaluation of said peak could be used for very early prognosis of a potential risk of an individual for developing cancer. Should this prove true everyone having a genetic disposition of developing malign tumors or cancer could, at a very early stage already, start monitoring his or her risk of falling ill of cancer by checking for the presence and size of said "cancer marker" peak at regular intervals.

10

15

While the above experiments have been carried out using whole blood samples it is within the scope of the present invention to retrieve analogous information from individuals by taking and using samples of other bodily fluids such as, for example saliva or sweat samples.

CLAIMS

1. Method of detecting a pathological condition, particularly the presence of a malignancy, more particularly of a malignant tumor or a cancer disease, in an individual, the method comprising:
- obtaining a sample of a bodily fluid from said individual;
 - obtaining a cell-free liquid fraction of said sample;
 - subjecting said cell-free fraction to analytical determination of at least one amino acid and/or amino acid related compound, preferably of a plurality of amino acids and/or amino acid related compounds, present in said fraction; and
 - evaluating qualitative and/or quantitative results of said analytical determination against corresponding values obtained from control samples derived from healthy blood donors,
- wherein statistically significant qualitative and/or quantitative differences in the values of said individual as compared to the controls indicates a pathological condition, particularly the presence of a malignancy, more particularly of a malignant tumor or of cancer cells or tissues.
2. The method according to claim 1, wherein said sample is selected from the group consisting of a whole blood sample, a saliva sample, and a sweat sample.
3. The method according to claim 1 or 2, comprising separating said cell-free liquid fraction into single compound fractions by liquid chromatography, preferably by HPLC, and detecting at least one, preferably a plurality of compound fractions, present in said cell-free fraction, using a suitable detection system.
4. The method according to claim 3, which comprises detecting in the control sample at least one compound fraction which is not detected, or only

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at a significantly lower concentration, in the sample obtained from said individual.

5. The method according to claim 4, wherein said significantly lower
5 concentration is in a range of equal or less than 20 %, preferably equal or less than 10%, of a pooled control.

6. The method according to claim 4 or 5, wherein under the analytical
10 conditions referred to in Example 1 at least one of said compound fractions present in the control sample but not or at a significantly lower concentration in the sample of said individual elutes at a retention time prior to aspartic acid.

7. The method according to claim 6, wherein under the analytical
15 conditions referred to in Example 1 one of said compound fractions present in the control sample but not or at a significantly lower concentration in the sample of said individual elutes at a retention time of about 2.5 min and/or another one at about 3 min and/or another one at about 3.7 min,, while aspartic acid elutes at about 4.3 min.

20

8. The method according to any one of claims 1 to 7, for early prognosis on the risk of cancer development in a said individual.

9. The method according to any one of claims 4 to 7, wherein the
25 detection of a compound fraction present in the sample of a said individual at a concentration significantly lower than in a pooled control is indicative of an increased risk of developing cancer or of the presence of an early stage cancer in said individual.

30 10. The method according to any one of claims 4 to 7, wherein a failure to detect in the sample of a said individual a compound fraction present in a

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pooled control is indicative of the presence of a malignant tumor or of cancer cells or tissues in said individual.

11. The method of claims 9 and 10, wherein said compound fraction
5 comprises a compound that elutes prior to aspartic acid under the analytical conditions referred to in Example 1.

12. The method according to claim 1, which comprises detecting at least
10 one compound fraction present in the experimental sample and not in a pooled control sample.

13. The method according to claim 12, wherein under the analytical
15 conditions referred to in Example 1 at least one of said compound fractions present in the experimental sample and not in the control sample elutes at a retention time later than that of alanine.

14. The method according to claim 13, wherein under the analytical
20 conditions referred to in Example 1 one compound fraction present in the experimental sample and not in the control sample elutes at a retention time of about 50 min and/or another one compound fraction present in the experimental sample and not in the control sample elutes at a retention time of about 70 min.

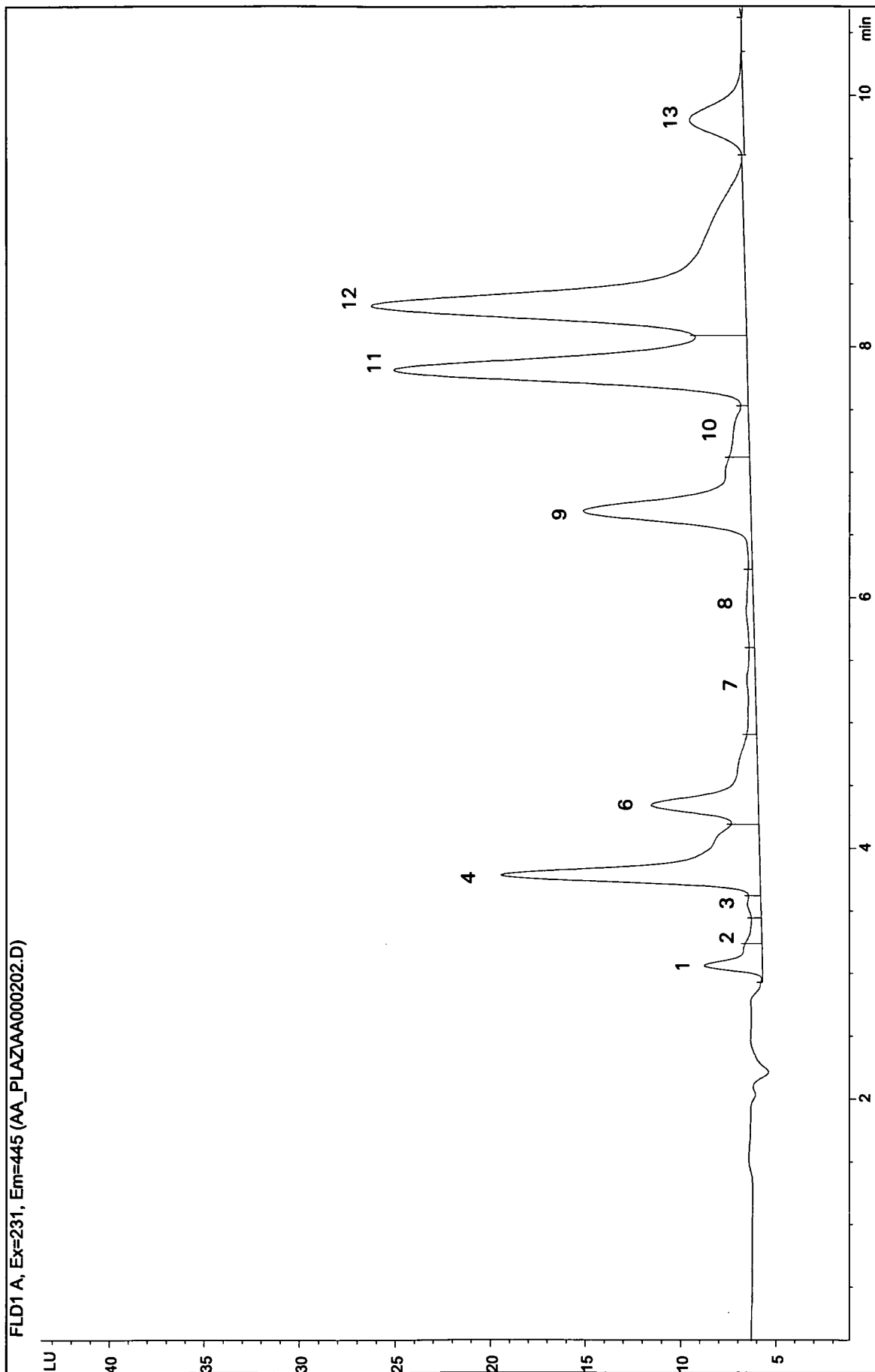


Fig. 1

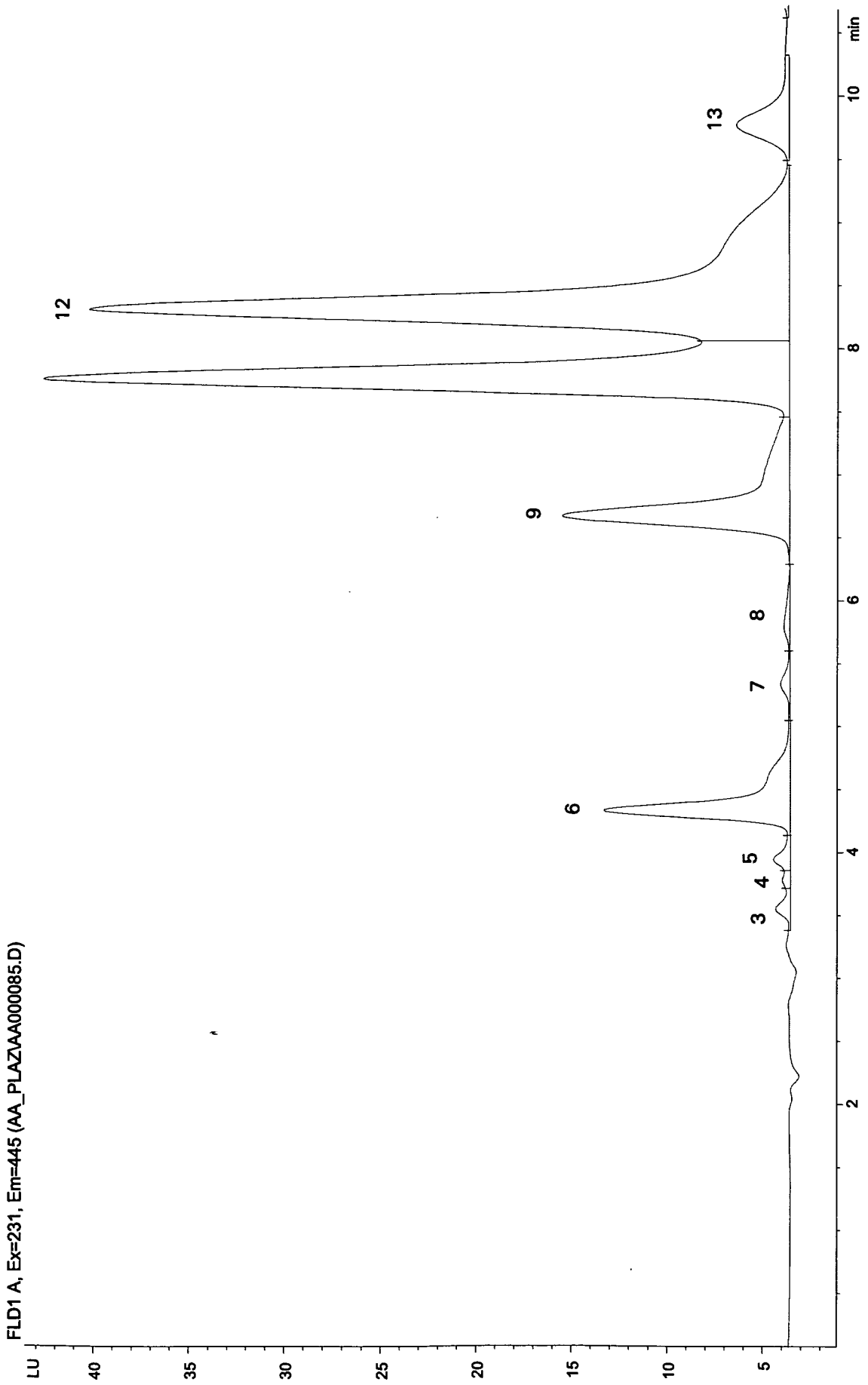


Fig. 2

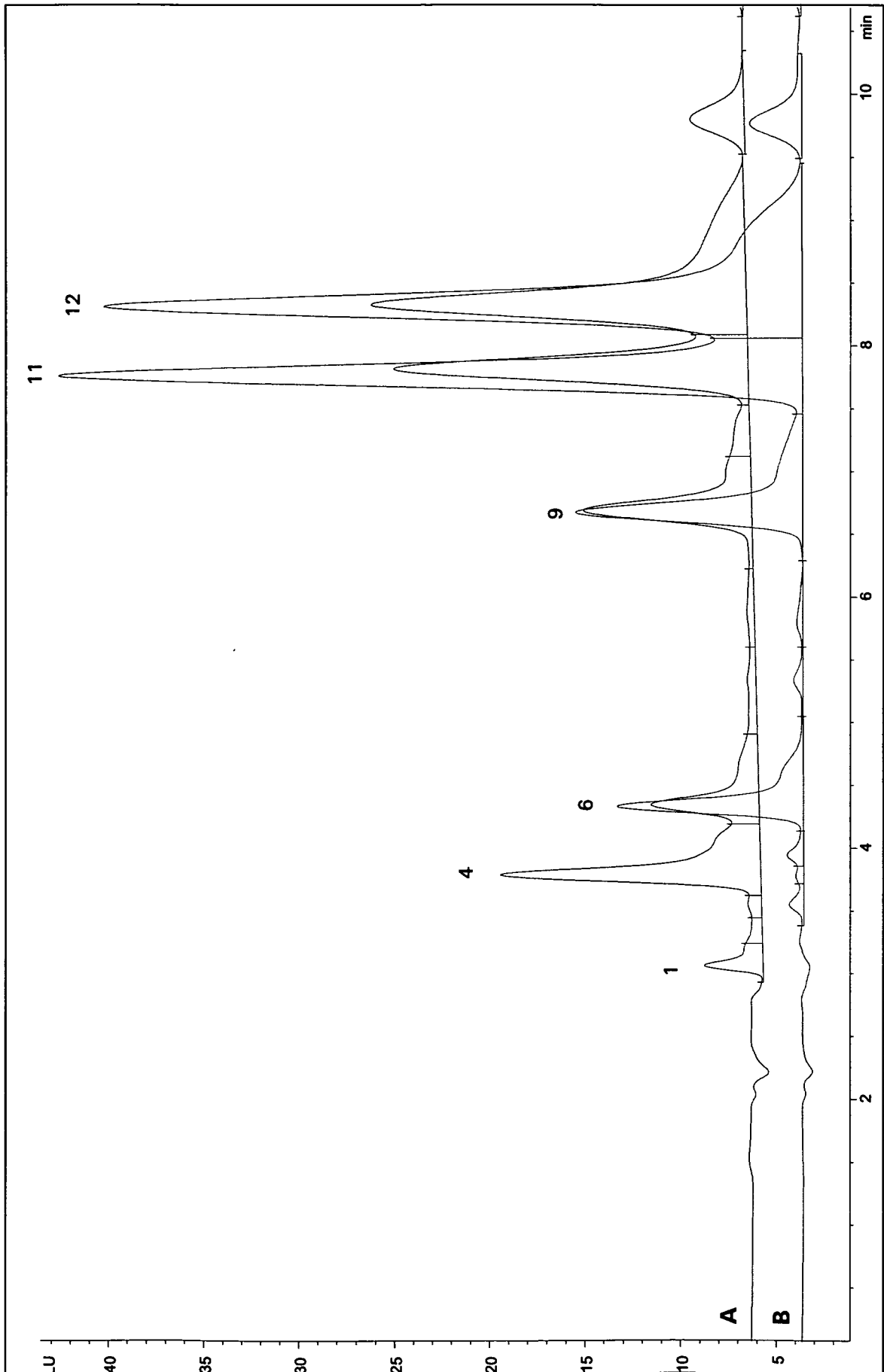


Fig. 3

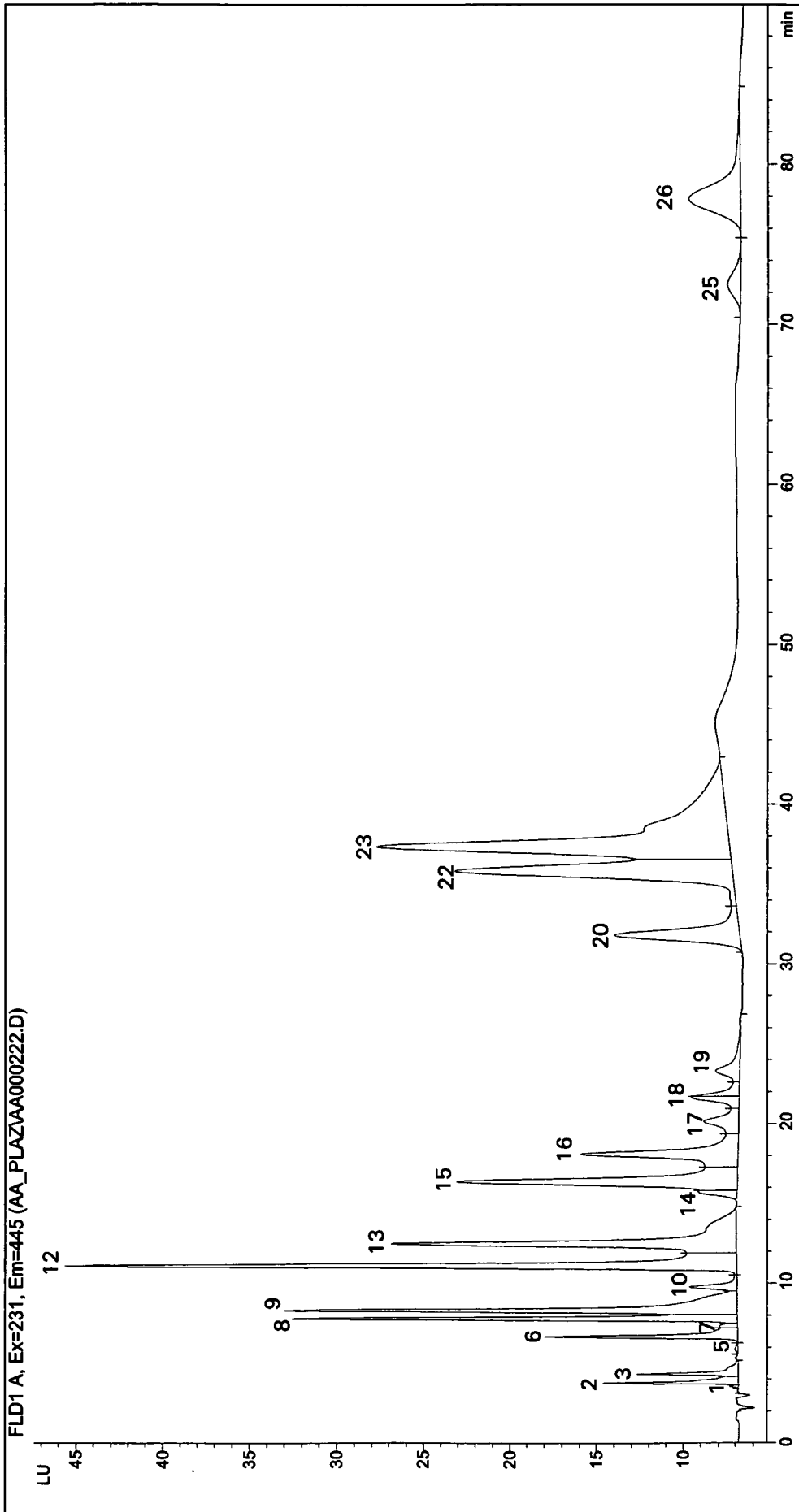


Fig. 4

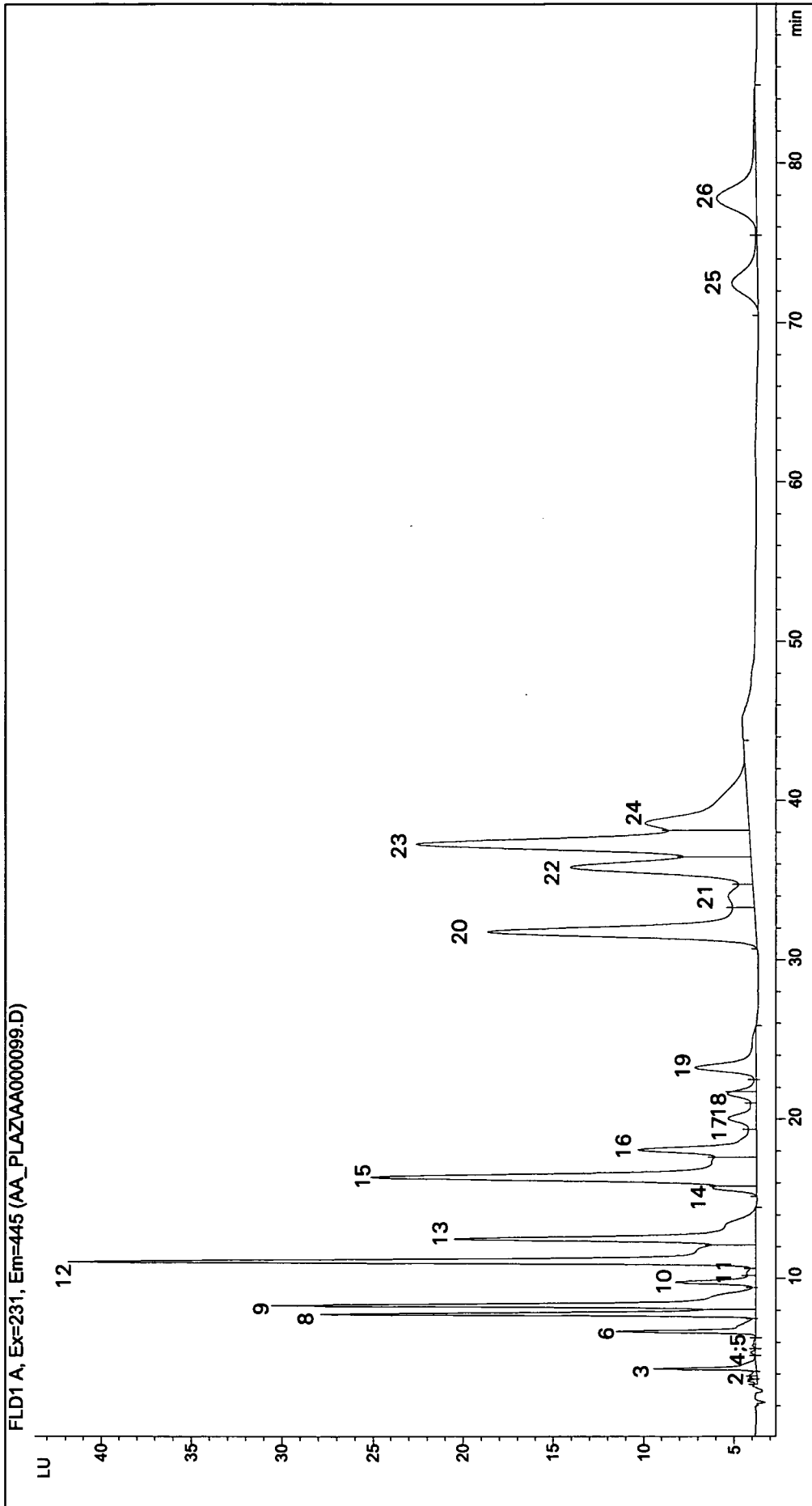


Fig. 5

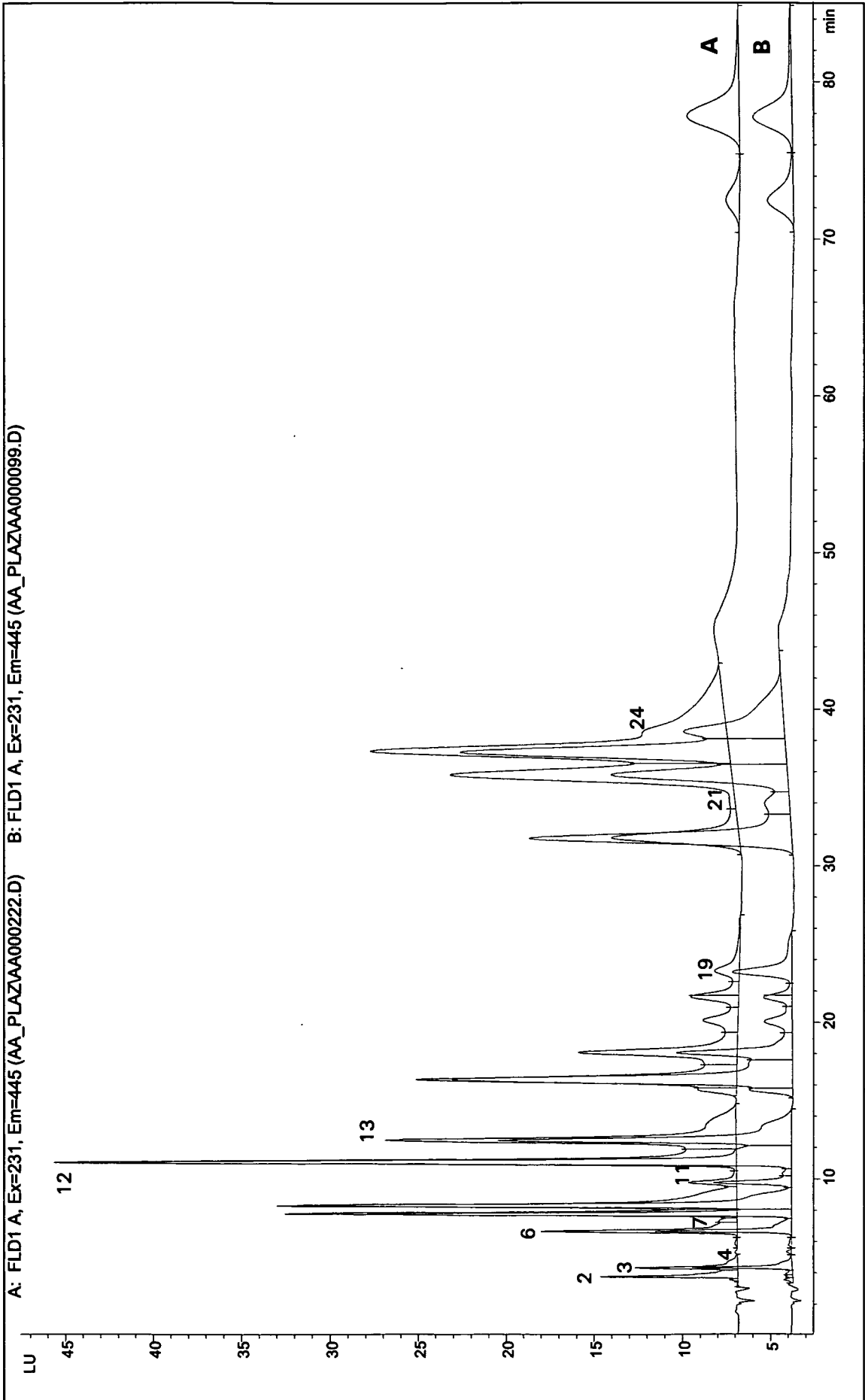


Fig. 6

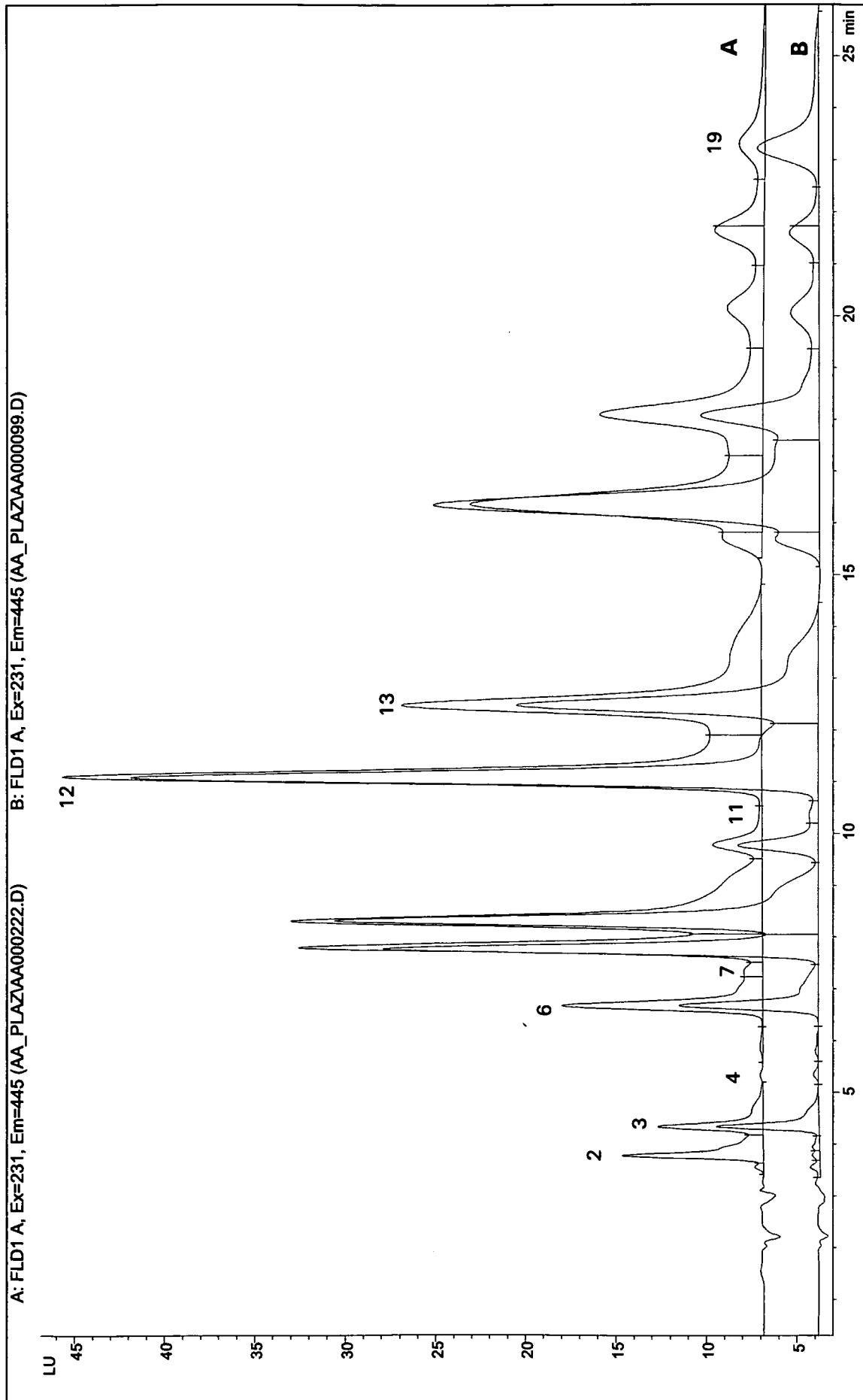


Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2007/002461A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574

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)
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 practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 2006/053680 A (NOWICKY WASSYL [AT]) 26 May 2006 (2006-05-26) examples 2,3	1-3,8,12
X	----- US 4 444 890 A (BURZYNSKI STANISLAW R [US]) 24 April 1984 (1984-04-24) claims 9-16 column 5, line 12 - column 7, line 18	1-5, 8-10,12
Y	column 5, line 12 - column 7, line 18 ----- -/--	6,7,11, 13,14

 Further documents are listed in the continuation of Box C. 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

E earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

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

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

31 May 2007

Date of mailing of the international search report

14/06/2007

Name and mailing address of the ISA/

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Authorized officer

Graf, Eva

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/002461

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 25 February 2003 (2003-02-25), VENKATAKRISHNA K ET AL: "HPLC-LIF for early detection of oral cancer." XP002435061 Database accession no. PREV200400168160 the whole document	1-5, 8-10,12
X	& CURRENT SCIENCE (BANGALORE), vol. 84, no. 4, 25 February 2003 (2003-02-25), pages 551-557, ISSN: 0011-3891 page 551, right-hand column page 552, right-hand column figures 3-5	1-5, 8-10,12
Y	page 552, right-hand column	6,7,11, 13,14
X	----- XIAOHUI FAN ET AL: "Diagnosis of breast cancer using HPLC metabonomics fingerprints coupled with computational methods" ENGINEERING IN MEDICINE AND BIOLOGY SOCIETY, 2005. IEEE-EMBS 2005. 27TH ANNUAL INTERNATIONAL CONFERENCE OF THE SHANGHAI, CHINA 01-04 SEPT. 2005, PISCATAWAY, NJ, USA,IEEE, 1 September 2005 (2005-09-01), pages 6081-6084, XP010907223 ISBN: 0-7803-8741-4 page 6082 - page 6083	1-5, 8-10,12
Y	page 6082	6,7,11, 13,14
X	----- JP 61 126472 A (ADVANCE RES & DEV CO LTD) 13 June 1986 (1986-06-13)	1-4, 8-10,12
Y	abstract	6,7,11, 13,14
Y	----- UGLYANITSA K N ET AL: "BIOCHEMICAL MECHANISMS OF THE ANTICANCER EFFECT OF UKRAIN IN THE TREATMENT OF CANCER OF THE URINARY BLADDER" INTERNATIONAL JOURNAL OF NUCLEAR MEDICINE AND BIOLOGY, PERGAMON PRESS, OXFORD, GB, vol. 29, no. 1, 2001, pages 79-84, XP008079186 ISSN: 0047-0740 page 80	6,7,11, 13,14
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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/002461

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>NEFYODOV L I ET AL: "AMINO ACIDS AND THEIR DERIVATIVES IN TUMOUR TISSUE FROM PATIENTS WITH BREAST CANCER TREATED WITH UKRAIN. PART VI" DRUGS UNDER EXPERIMENTAL AND CLINICAL RESEARCH, BIOSCIENCE EDIPRINT INC, XX, vol. 12, no. 3-5, 1996, pages 159-161, XP008060488 ISSN: 0378-6501 page 160</p>	6,7,11, 13,14
A	<p>----- DATABASE MEDLINE [Online] US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US; October 1987 (1987-10), HIRAYAMA C ET AL: "Plasma amino acid patterns in hepatocellular carcinoma." XP002435062 Database accession no. NLM2823852 the whole document & BIOCHEMICAL MEDICINE AND METABOLIC BIOLOGY OCT 1987, vol. 38, no. 2, October 1987 (1987-10), pages 127-133, ISSN: 0885-4505</p>	1-14
A	<p>----- NEFYODOV L I ET AL: "AMINO ACIDS AND THEIR DERIVATIVES IN BLOOD PLASMA OF PATIENTS WITH BREAST CANCER TREATED WITH UKRAIN. PART V" DRUGS UNDER EXPERIMENTAL AND CLINICAL RESEARCH, BIOSCIENCE EDIPRINT INC, XX, vol. 22, no. 3 - 5, 1996, pages 155-157, XP008060480 ISSN: 0378-6501 the whole document</p>	1-14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2007/002461

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 1-14 [partially]
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(iv) PCT - Diagnostic method practised on the human or animal body
2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2007/002461

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 2006053680	A	26-05-2006	AR	051502 A1	17-01-2007
US 4444890	A	24-04-1984	NONE		
JP 61126472	A	13-06-1986	NONE		