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Datasheet for the decision of 18 July 2017

Case Number: T 1137/11 - 3.3.08

Application Number: 03777537.6

Publication Number: 1556478

IPC: C12N1/06

Language of the proceedings: EN

Title of invention:
HIGH SENSITIVITY QUANTITATION OF PEPTIDES BY MASS SPECTROMETRY

Applicant:
Anderson, Norman Leigh

Headword:
Quantitation of peptides/ANDERSON

Relevant legal provisions:
EPC Art. 54, 83, 84, 111(1), 123(2)

Keyword:
Main request - added matter (no)
Novelty - document (5) - (yes)
Remittal (yes)

Decisions cited:

Catchword:

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Case Number: T 1137/11 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 18 July 2017

Appellant: (Applicant)	Anderson, Norman Leigh 1759 Willard Street, NW Washington, DC 20009 (US)
Representative:	Bohmann, Armin K. Bohmann Anwaltssozietät Nymphenburger Straße 1 80335 München (DE)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 22 November 2010 refusing European patent application No. 03777537.6 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman	B. Stolz
Members:	M. R. Vega Laso
	J. Geschwind

Summary of Facts and Submissions

I. The appeal lies from a decision of an examining division of the European Patent Office posted on 22 November 2010 refusing the European patent application no. 03 777 537.6 under Article 97(2) EPC. The application with the title "High sensitivity quantitation of peptides by mass spectrometry" was filed under the Patent Cooperation Treaty and published as WO 2004/031730 (in the following "the application as filed").

II. In the decision under appeal, the examining division found that the subject-matter of the claims according to the main request and auxiliary requests 1 and 2 then on file extended beyond the content of the application as filed and thus offended against Article 123(2) EPC, and that the subject-matter of the claims according to auxiliary request 3 lacked novelty in view of document (5).

III. Together with its statement of grounds of appeal, the appellant submitted additional evidence and five sets of amended claims as main request and auxiliary requests 1 to 4 which replaced the requests underlying the decision under appeal. As a subsidiary request, the appellant requested oral proceedings.

IV. The board summoned the appellant to oral proceedings. In a communication sent in preparation of the oral proceedings, the board expressed a provisional opinion on the findings in the decision under appeal concerning Articles 123(2) and 54 EPC, and raised new objections under Articles 123(2) and 84 EPC.

V. Oral proceedings were held on 18 July 2017. At the outset of the proceedings, the appellant withdrew the requests filed together with the statement of grounds of appeal, and filed two sets of amended claims as new main and auxiliary request, respectively.

VI. Claims 1 and 2 of the main request read as follows:

"1. A method for quantifying the amount of a peptide in a biological sample, comprising:
contacting the biological sample with
(i) an anti-peptide antibody specific for said peptide;
(ii) a known quantity of an isotopically labeled version of the peptide;
separating peptide bound by said antibody from unbound peptide;
eluting said peptide and said isotopically labeled version of the peptide bound by said antibody from said antibody;
measuring the amount of the peptide and said isotopically labeled version of said peptide eluted from said antibody using a mass spectrometer; and
calculating the amount of the peptide in the biological sample,
wherein said biological sample is a proteolytic digest of a body fluid.

2. The method of claim 1, wherein the labeled version of the peptide includes at least one site at which a stable isotope is substituted for the predominant natural isotope in more than 98% of peptide molecules, wherein the stable isotope is selected from the group consisting of ^{15}N and ^{13}C."

Dependent claims 3 to 6 are directed to particular variants of the method of claim 1.

VII. The following document is referred to in the present decision:

(5): US 2002/0037532 A1, published on 28 March 2002.

VIII. The submissions made by the appellant concerning issues relevant to this decision, were essentially as follows:

Article 123(2) EPC

The amendments to claims 1 to 6 did not add any subject-matter which extended beyond the content of the application as filed and thus complied with Article 123(2) EPC.

Article 54 EPC - Document (5)

The subject-matter of claims 1 to 6 was novel in view of document (5). The methods described in this document were intended mainly to allow comparison of relative changes in concentration of many proteins between different samples. This document described a discovery technology and did not teach or suggest methods for determining the absolute concentration of single specific peptides in complex peptide digests.

IX. The appellant (applicant) requested that the decision under appeal be set aside and that the case be remitted to the examining division for further prosecution on the basis of the main request filed during the oral proceedings.

Reasons for the Decision

Admission of the new requests into the proceedings

1. The amendments introduced into the sets of claims according to the main request and auxiliary request filed at the oral proceedings are a clear reaction to objections under Articles 123(2) and 84 EPC raised for the first time in the board's communication. As the amendments are straightforward (deletion of claims 1 to 7 and 13, and amendments to claims 8 and 9 of the main request as submitted together with the statement of grounds of appeal), and do not give rise to new objections, the new sets of claims are admitted into the proceedings.

Article 123(2) EPC

2. In the decision under appeal, the examining division did not raise any objection under Article 123(2) EPC in respect of independent claim 9 of the main request then on file. Present claim 1 differs from the previous claim 9 in that (i) the sample containing the peptide to be quantified is characterized as a biological sample, and (ii) the labeled version of the peptide which is contacted with the biological sample is specified as being isotopically labeled.

3. Basis for amendment (i) is found, *inter alia*, in the first paragraph of the description which reads:

> "*This invention relates to quantitative assays for evaluation of proteins in complex samples such as human plasma. The invention can be used both for the analysis of samples from a single individual source or, for purposes of evaluating the level of*

a particular protein in a population, can be used to analyze pooled samples from the target population."

While a "*biological sample*" is not expressly mentioned in this passage, it is immediately apparent to a skilled person in the field of protein analysis that a complex sample containing proteins which is obtained from an individuum or a population must be a biological sample, such as human plasma.

4. Basis for an isotopically labeled version of the peptide to be quantified (amendment (ii)) is found, *inter alia*, in the paragraph under the heading "Summary of the invention" on page 8 of the application as filed. In particular, in lines 25 to 27 it is stated:

"*Upon elution into a suitable mass spectrometer, the natural (sample derived) and internal standard (isotope labeled) peptides are quantitated,...*"

5. Claims 1 to 6 according to the present main request correspond to, respectively, claims 9, 10, 12 to 14 and 16 of the main request underlying the decision under appeal. Thus, except for claim 10, from which present claim 2 is derived, the claims objected to by the examining division under Article 123(2) EPC (claims 5, 8 and 11 of the main request then on file) have been deleted in the set of claims according to the main request now on file.

6. Present claim 2 (see section VI above) differs from the previous claim 10 in that the isotopically labeled version of the peptide used in the claimed method includes a stable isotope selected from the group consisting of ^{15}N and ^{13}C that replaces the predominant

natural isotope in more than 98% of the labeled peptide molecules. This feature has a basis in the paragraph under the heading "Creating isotope monitor peptides (step b)" on page 17 of the application as filed. The relevant passages of this paragraph read as follows:

> "*An isotopically labeled version of the selected peptide(s) is then made in which the chemical structure is maintained, but one or more atoms are substituted with an isotope such that an MS can distinguish the labeled peptide from the normal peptide (containing the natural abundance of each elements' isotopes). For example, nitrogen-15 could be introduced instead of the natural nitrogen-14 at one or more positions in the synthesized peptide. [...] ln the preferred embodiment, nitrogen-15 labeled amino acid precursors substituted at >98% are used at one or more positions in the peptide synthesis process [...]. Such nitrogen-15 labeled amino acid precursors (or their carbon-13 labeled equivalents) are commercially available as FMOC derivatives suitable for use directly in conventional commercial peptide synthesis machines.*"

7. The board is thus satisfied that the amendments introduced into the claims of the main request do not add subject-matter which extends beyond the content of the application as filed. Article 123(2) EPC is complied with.

Articles 84 and 83 EPC

8. No objections under Articles 84 and 83 EPC were raised in the decision under appeal, and the board does not see any reason to raise any of its own motion.

Article 54 EPC – Document (5)

9. In section 2.1 of the decision under appeal, the examining division, after referring to various passages of document (5) that purportedly disclosed particular aspects of the invention as claimed, found that this document anticipated the subject-matter of claims 1 and 8 of auxiliary request 3. Present claim 1 is, except for the two amendments specified in paragraph 5 above, identical to claim 8 of the auxiliary request 3 underlying the decision under appeal.

10. The board does not share the examining division's view concerning the novelty of the claimed subject-matter in view of document (5). This document describes numerous methods for identification and quantification of proteins in complex mixtures which utilize isotopically labeling and affinity selection of tryptic peptide fragments as analytical surrogates for the proteins (see paragraph [0013]). As used in document (5), "quantification" is to be understood as the measurement of either relative changes in protein/peptide concentration between two different samples, or the relative abundance of a protein/peptide within a sample (see paragraph [0012]).

11. Various methods for detecting a difference in the concentration of a protein present in a first sample and in a second sample are defined in independent claims 1, 4, 5, 6 and 33. These methods comprise isotopically labeling the peptides of the first sample with a first isotope and the peptides of the second sample with a second isotope, by attaching a labeled chemical moiety to the peptides. The samples are mixed together to yield a combined sample, which is subjected

to mass spectrometric analysis (see paragraph [0025]). In one embodiment, the combined peptide sample is subjected to a fractionation step, for example using a chromatographic or electrophoretic technique, in order to reduce the complexity of the mixture prior to the determination of peptide masses. For that purpose, the members of at least one pair of chemically equivalent, isotopically distinct peptides include at least one affinity ligand, which can be endogenous (e.g. specific amino acids present in the peptide, for instance cysteine or histidine) or exogenous, i.e. attached to a protein or peptide in the sample before or after proteolytic cleavage (see paragraphs [0026] and [0054]).

12. The methods defined in claims 59 to 62 are aimed at quantifying the (relative) amount of two or more different peptides which have very similar or even identical mass and chromatographic separation properties ("isobaric peptides"). These methods require a second dimension of mass spectrometry in order to resolve the fragment ions generated during gas phase fragmentation of the peptide in the first mass spectrometry (see paragraphs [0036] and [0037] and Example IX).

13. None of the various methods described in document (5) is aimed at measuring the **absolute amount** of a peptide in a sample, as it is the method of the present invention. This is confirmed by the following statements in paragraph [0105] of document (5):

> "*A key advantage of the isotope labeling method of the invention is that it detects* ***relative*** *change, not changes in absolute amounts of analytes. It is very difficult to determine changes in absolute*

amounts analytes that are present at very low levels." (emphasis added by the board)

14. Further, while it is suggested in document (5) that a portion of the protein or peptide amino acid sequence that defines an antigen can also serve as an endogenous affinity ligand (see paragraph [0069]), a method using an anti-peptide antibody **specific for** the peptide to be quantified cannot be derived, directly and unambiguously, from this document. On the contrary, it is stated in paragraph [0069] that "*... [it] is particularly useful if the endogenous amino acid sequence is common to* ***more than one protein*** *in the original mixture*" (emphasis added by the board).

15. Furthermore, document (5) does not describe a method using **a known quantity** of an isotopically labeled version of the peptide to be quantified. The board disagrees with the finding in the decision under appeal that paragraphs [0020], [0097], [101] and [102] disclose the use of an internal standard in a method for quantifying a peptide as defined in claim 1. While there is no reference whatsoever to an internal standard in paragraph [0020], the relevant passages of paragraphs [0097], [101] and [102], which in fact relate to internal standard quantification, read:

"[0097] Internal standard quantification with signature peptides
.....
[0101] The internal standard method of quantification is based on the concept that the concentration of an analyte (A) in a complex mixture of substances may be determined by adding a known amount of a very similar, but distinguishable

substance (Λ) to the solution and determining the concentration of A relative to Λ. [...]

[0102] The term Δ is the relative concentration of A to that of the internal standard Λ and is widely used in analytical chemistry for quantitative analysis. It is important that A and Λ are as similar as possible in chemical properties so that they will behave the same way in all the steps of the analysis. It would be very undesirable for A and Λ to separate. One of the best ways to assure a high level of behavioral equivalency is to isotopically label either the internal standard (Λ) or the analyte (A)."

16. The examining division failed, however, to consider the following statements in paragraphs [0103] and [0104]:

"*[0103] ... The internal standard method apparently cannot be applied here because i) the analytes* A_{1-n} *undergoing change are of unknown structure and ii) it would be difficult to select internal standards* Λ_{1-n} *of nearly identical properties.*

[0104] Post-synthetic isotope labeling of proteins in accordance with the method of the invention advantageously creates internal standards from proteins of unknown structure and concentration. Whenever there is a control, or reference state, in which the concentration of proteins is at some reference level, proteins in this control state can serve as internal standards."

17. Hence, document (5) describes an internal standard method of quantification in general terms, but also indicates that this method cannot be applied to complex

mixtures. Instead, document (5) suggests methods for identifying analytes that have changed in concentration between two samples, which comprise (i) mixing together the two isotopically labeled samples or at least a fraction of each that contains the desired components selected from the mixture, ii) subjecting the combined sample to mass spectrometric analysis, and (iii) determining a concentration ratio between the peptide amounts in each of the two samples (see paragraph [0025]). When the method involves labeling all peptide fragments, it is referred to in document (5) as the global internal standard technology (GIST) method (see Figure 1), in which components from control samples function as standards against which the concentration of components in experimental samples are compared. It is clear from paragraph [0028] that, applying this technology, only the **relative** concentration of all components in complex mixtures can be quantified.

18. For these reasons, the method of claim 1 as well as those of dependent claims 2 to 6 are considered to be novel in view of document (5). Since the examining division's finding of lack of novelty over this document is incorrect, the decision under appeal must be set aside, as requested by the appellant.

Remittal for further prosecution

19. The decision under appeal did not deal with either the issue of novelty in view of other documents on file - other than document (5) – or the issue of inventive step. The board, exercising its discretion under Article 111(1) EPC, decides to remit the case to the examining division for further prosecution, as requested by the appellant.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the examining division for further prosecution on the basis of the main request (claims 1 to 6) filed during the oral proceedings.

The Registrar: The Chairman:

A. Wolinski B. Stolz

Decision electronically authenticated