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# D E C I S I O N
## of 18 September 2001

**Case Number:** T 0339/98 - 3.3.4

**Application Number:** 89310061.0

**Publication Number:** 0363126

**IPC:** C07K 3/20

**Language of the proceedings:** EN

**Title of invention:**
Method for the purification of vitamin K-dependent proteins

**Patentee:**
ELI LILLY AND COMPANY

**Opponent:**
Baxter Aktiengesellschaft

**Headword:**
Vitamin K-dependent proteins/ELI LILLY

**Relevant legal provisions:**
EPC Art. 123(2), 56

**Keyword:**
"Main request - added matter (yes)"
"Auxiliary request - inventive step (no)"

**Decisions cited:**
T 0596/96, T 0863/96, T 0597/92, T 0917/94, G 0009/92

**Catchword:**
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Case Number: T 0339/98 - 3.3.4

# D E C I S I O N
## of the Technical Board of Appeal 3.3.4
## of 18 September 2001

**Appellant:** (Proprietor of the patent)
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**Decision under appeal:** **Interlocutory decision of the Opposition Division of the European Patent Office posted 4 February 1998 concerning maintenance of European patent No. 0 363 126 in amended form.**

**Composition of the Board:**

**Chairman:** U. M. Kinkeldey
**Members:** L. Galligani
S. U. Hoffmann

## Summary of Facts and Submissions

I. The patent proprietors lodged an appeal against the interlocutory decision of the opposition division issued 4 February 1998 whereby the European patent No. 0 363 126 with title "Method for the purification of vitamin K-dependent proteins", which had been opposed by one party on grounds of Article 100(a) EPC (lack of novelty and lack of inventive step), was maintained in amended form on the basis of the auxiliary request then on file. Claim 1 therein read as follows:

" A method for recovering and purifying vitamin K-dependent proteins from a cell culture medium of cells which produce vitamin K-dependent proteins, said medium containing forms of the desired vitamin K-dependent protein that differ in γ-carboxyglutamate content and therefore in specific activity, said method comprising:

a. removing divalent cations from the medium;

b. contacting the medium with a protein-binding ion-exchange resin under conditions such that the protein is bound to the resin;

c. treating the resin-bound protein with a divalent cation under conditions appropriate to form a cation-protein complex and to thereby dissociate the high specific activity vitamin K-dependent protein from the resin while leaving lower specific activity vitamin K-dependent protein bound to the resin; and

d. treating the dissociated cation-protein complex under conditions appropriate to remove the cation to obtain free, biologically active protein."

II. The opposition division decided that the subject-matter of the main request then on file lacked an inventive step having regard in particular to the following document:

(1) Grinnell B.W. et al., BIO/TECHNOLOGY, Vol. 5, November 1987, pages 1189 to 1192.

The subject-matter of the auxiliary request was considered to involve an inventive step.

III. With the statement of grounds of appeal on 12 June 1998, the appellants filed a new main request and two auxiliary requests, the second one being to maintain the patent on the basis of the claims as accepted by the opposition division.

Claim 1 of the **main request** (claims 1 to 33) read as follows (in bold-type letters the difference in comparison with claim 1 as granted):

" A method for recovering and purifying vitamin K-dependent proteins from a cell culture medium of transformed cells which produce recombinant vitamin K-dependent proteins, comprising:

a. removing divalent cations from the medium;

b. contacting the medium with a protein-binding ion-exchange resin under conditions such that the protein is bound to the resin;

c. treating the resin-bound protein with a divalent cation under conditions appropriate to form a cation-protein complex and to thereby dissociate the protein from the resin; and

d. **without further purification of the cation-protein complex with an immobilized antibody to said cation-protein complex,** treating the cation-protein complex under conditions appropriate to remove the cation to obtain free, biologically active protein."

As for the remaining claims: dependent claims 2 to 6 concerned embodiments of the method of claim 1; independent claim 7 (together with dependent claims 8 to 16), independent claim 17 (together with dependent claims 18 to 21), independent claim 24 (together with dependent claims 25 and 26), independent claim 27 (together with dependent claim 28) were directed to particular variations of the method in which steps (a) to (i) were specified; independent claim 22 (together with dependent claim 23), independent claim 29 (together with dependent claims 30 and 31), independent claim 32 (together with claim 33) were directed to methods comprising the same steps (a) to (d) as in claim 1.

IV. In their reply to the statement of grounds of appeal, the respondents made submissions raising inter alia objections under Article 123(2) EPC, and disputing the inventive step of the main and first auxiliary requests.

V. On 28 February 2001, the board issued a communication with an outline of the points to be discussed and a provisional, non-binding opinion on some of the issues.

VI. In reply thereto, submissions were made by the appellants with letter dated 4 May 2001. Therewith a new first auxiliary request (claims 1 to 32) was filed in substitution of the previous one.

Claim 1 thereof read as follows (in bold-type letters the differences in comparison with claim 1 as granted):

" A method for recovering and purifying vitamin K-dependent proteins from a cell culture medium of transformed cells which produce recombinant vitamin K-dependent proteins, comprising:

a. removing divalent cations from the medium;

b. contacting the medium with a protein-binding ion-exchange resin under conditions such that the protein is bound to the resin;

c. treating the resin-bound protein with a divalent cation under conditions appropriate to form a cation-protein **complex**, [**and**] to thereby dissociate the **cation-protein complex** from the resin **and form resin dissociated cation-protein complex**; and

d. treating the **resin-dissociated** cation-protein complex **with a chelating agent** under conditions appropriate to remove the cation to obtain free, biologically active protein."

The remaining claims corresponded to the claims of the main request, due account being taken of the renumbering in consequence of the introduction of the features of claim 6 into claim 1.

VII. Oral proceedings took place on 4 July 2001.

VIII. The appellants submitted essentially that:

(a) As regards the feature "without further purification... " in item d) of claim 1 of the main request:

- The feature had to be seen as a disclaiming feature which had a basis in the application as filed because the complete description of the latter made abundantly clear that conventional chromatography, **not** immunoaffinity chromatography was used for the separation of the subject proteins (cf the published "A2" application, page 2, lines 43 to 50; page 3 lines 43 to 44 and page 4 lines 49 to 50; Example 12). Immunoaffinity chromatography was not a conventional chromatography;

- The removal of the divalent cations from the cation-protein complex in step d) was achieved only by use of chelating agents, in particular immobilised chelating agents, **not** by way of immunochromatography.

- From the disclosure of the original documents as a whole the skilled person would not have inferred any form of use of monoclonal antibodies. It was thus legitimate to specify that in the claim.

(b) As for the feature "resin-dissociated" in claim 1 of the auxiliary request, the term was clear and unambiguous as a cation-protein complex which was dissociated from a resin as originally disclosed had necessarily to be a "resin-dissociated" cation-protein complex.

(c) As regards inventive step of the subject-matter of the auxiliary request:

The key element of the disclosure of document (1) was the use of a specific monoclonal antibody for purifying recombinant human protein C (HPC) (see the abstract). It was found that the anti-HPC immunoaffinity column bound HPC only in the presence of calcium ions (cf page 1192, left-hand column, lines 31 to 32). To this extent, in the preliminary purification steps described in the small print of the article calcium chloride was added to the eluent in order to elute the protein from the ion-exchange resin in the form of a calcium-HPC complex to be applied to the anti-HPC column in the next step. This was the only explanation for the addition of calcium chloride which the skilled reader could derive from document (1). In view of the importance attributed in document (1) to the immunoaffinity step, and also in consideration of the fact that no data were reported of any degree of purification in the steps preceding it, there was no reason for the skilled reader to stop the purification process of HPC just before the immunoaffinity step and to proceed to the removal of the calcium ions, and there would have been no apparent reason for adding calcium ions in the eluent of the ion-exchange column if these were to be removed after elution.

The method of the claims at issue consisted in a series of steps using conventional chromatography in which the removal, the addition and the subsequent chelation of the divalent cation was **knowingly** devised in order to achieve a high degree of purification, avoiding thereby the use

of immunoaffinity. This was something that the authors of document (1) had not recognised, and which the skilled person could not readily derive from the disclosure of document (1).

IX. The respondents argued as follows:

(a) As regards the feature "without further purification..." in item d) of claim 1 of the main request:

- The feature in question had been introduced as a disclaimer to restore novelty vis-à-vis document (1);

- The case law specified the circumstances in which a disclaimer could be allowed, namely that no disclaimer should be used when a definition by positive features was possible, that a disclaimer had to be used only for restoring novelty vis-à-vis an accidental disclosure and that the document on the basis of which a disclaimer was construed had to disappear from the state of the art for the purpose of the discussion of inventive step (cf eg T 596/96 of 14 December 1999; T 863/96 of 4 February 1999). In the case at issue, a definition of the claimed subject-matter in positive terms was possible. Moreover, document (1) was not an accidental disclosure. As a matter of fact it was relevant for the discussion of inventive step. Thus, the amendment could not be allowed.

(b) As for the feature "resin-dissociated" in claim 1 of the auxiliary request, it had no explicit basis in the application as filed and was not derivable therefrom.

(c) As regards inventive step of the subject-matter of the auxiliary request:

The skilled person would have derived from document (1) that after steps (a) to (c) there was already a sufficient degree of purity of HPC (cf page 1192). The document stated explicitly that the immunopurification step was a **further** purification step (cf page 1192, left-hand column, lines 45 to 47). Therefore, the skilled person would readily have had the idea of stopping after the first three steps which corresponded to steps (a) to (c) of claim 1, and would have obviously removed the calcium from the eluted protein by using a chelating agent, this being then the equivalent of step d) in claim 1. For these reasons, there was no inventive step involved in the claim.

X. The appellants requested that the decision under appeal be set aside and the patent be maintained on the basis of claims 1 to 33 filed on 12 June 1998 (main request) or claims 1 to 32 dated 4 May 2001 (auxiliary request).

The respondents requested that appeal be dismissed.

XI. At the end of the oral proceedings, the following decision was announced:

1. The debate is closed.

2. The decision will be given in writing.

## Reasons for the Decision

*The main request: Formal admissibility*

1. Claim 1 of this request differs from claim 1 as granted in that it contains the feature "without further purification...". Because of the restrictive nature of the feature, no problems under Article 123(3) EPC are seen by the board.

2. As also admitted by the appellants, the feature as such is not found in the application as filed. However, in the appellant's view, the feature can be inferred from the application as filed essentially because therein no use of monoclonal antibodies in any form is disclosed, all separations being based on conventional chromatography.

3. Although it is true that the application as filed does not make any specific explicit reference to the use of monoclonal antibodies at any stage of the purification process, it is also a fact that it does not explicitly exclude this possibility. As a matter of fact, claim 1 at issue is in the "comprising" form, which implies that, while steps (a) to (d) are the essential features of the claimed invention, the presence of other steps, including an immuno-chromatography step (eg between steps (c) and (d) is not excluded. Any variant process "comprising" steps (a) to (d) would in fact be encompassed by the claim. In the board's judgment, the statement in the specification (cf eg page 3, lines 43 to 44) that "the invention is based upon the use of conventional chromatography resins" does not amount to an absolute ban of immuno-chromatography because, firstly, the statement is in relation to the characterising essential steps which are "comprised" in the claimed invention (cf claim 1), and, secondly, it

is open to interpretation whether or not in 1988/89 immunoaffinity chromatography could be defined as a conventional chromatography. On this the two parties had, of course, divergent views.

4. Thus, in the board's view, the addition of the feature in question amounts to the presentation of new information, this being "not to do" was the skilled person would not have excluded. Such information is not directly and unambiguously derivable from the application as filed.

5. If the addition of the feature in question is considered as a disclaimer, it cannot be accepted as it is in contrast with the principles set by the established case law on "disclaimers", namely that a disclaimer is admissible **only** for excluding from the ambit of a claim, for the purpose of restoring novelty, an "accidental disclosure" by a prior art document, the said document not being relevant for the evaluation of inventive step (cf T 863/96 and T 596/96 supra, and T 917/94 of 28 October 1999; T 597/92 OJ EPO 1996, 135).

6. For the above reasons, claim 1 as well as all claims containing the same feature in question offend against Article 123(2) EPC. Thus, the main request fails to comply with the formal requirements and is thus not allowable.

*The first auxiliary request*

*Formal admissibility*

7. Claim 1 has a narrower scope of protection than claim 1 as granted as it specifies in step d) that the dissociation is carried out with a chelating agent (this being the feature of claim 6 as granted). Thus, no problems under Article 123(3) EPC are seen by the board.

8. As for the feature "resin-dissociated" which qualifies the cation-protein complex, in the board's judgement, although it is not found as such in the application as filed, it is nevertheless unambiguously implied therein by the fact that - as originally disclosed - the eluent in step c) "dissociates" the protein as a cation-protein complex (cf claim 1 as filed) and thus results in the formation of a "resin-dissociated" cation-protein complex. There is thus no objection under Article 123(2) EPC.

*Novelty (Article 54 EPC)*

9. Novelty of any of the claims of this request was not disputed by the respondents. Nor does the board have any novelty objection.

*Inventive step (Article 56 EPC)*

10. The closest prior art document is document (1). This document describes the expression of recombinant HPC (rHPC) in human kidney cell lines and its purification and biochemical analysis. The latter are described on page 1192, left-hand column under the heading "Purification and biochemical analysis of HPC" which also includes the description of a parallel procedure carried out on HPC from plasma. Both purification procedures included, as a key step for achieving homogeneity of the product, the passage through an immunoaffinity column having immobilised anti-HPC monoclonal antibody which was "conformation-specific"

as it bound to HPC only in the presence of calcium ions. Prior to this step, the procedure provided for addition of EDTA and passage through an ion-exchange resin. The resin used in the case of rHPC was different from that used for plasma HPC. Since in the case of the latter, the eluent did not contain calcium ions, the fraction containing the HPC had to be adjusted to 10mM $CaCl_2$. This addition was not necessary in the case of rHPC as the eluent already contained 10mM $CaCl_2$. Binding to and elution from the immunoaffinity column was carried out under the same conditions in both cases with a buffer containing EDTA. After this step, in both cases a further ion-exchange purification followed, the resins used being different. The document does not report any data concerning the level of purification at the different stages. However, in respect of rHPC, it is stated on page 1190, under the heading "Purification and characterization of rHPC": "The recombinant HPC secreted from this cell line was purified to homogeneity, with 85 to 90% recovery at each step", and on the same page under the heading "Functional analyses of rHPC": "The rHPC in the crude culture medium and through each step in purification was fully functional as measured by both its anticoagulant and amidolytic activities".

11. In the light of document (1), the technical problem underlying the present patent was finding an alternative method for recovering and purifying vitamin K-dependent proteins, eg HPC, from a cell culture medium.

12. The proposed solution is a method which comprises the four steps (a) to (d) recited in claim 1. Example 4 shows that the purity of the rHPC selectively eluted from an anion exchange column with 10mM $CaCl_2$ is increased of 232 fold, while that of the same product eluted in a "conventional" manner with 0.4M NaCl is

increased of 28 fold. It is noted that the operating conditions (type of column, eluent, specific activity determination) used in the experiment of Example 4 are similar to those described in document (1) to which explicit reference is made.

13. The relevant question is what measures would have been adopted by the skilled person faced with the stated technical problem and whether these would have included a method knowingly **comprising** the four steps (a) to (d) referred to in claim 1. The word "comprising" is emphasised because - as already noted above in connection with the main request - the method of claim 1 is broadly formulated and does not necessarily "consist" only of the said four steps. This is to be taken into account when answering the above question.

14. In the board's judgement, a method in which steps (a) to (d) would have been **knowingly** applied (cf claim 1), would have been an obvious option for the skilled person for the following reasons:

(i) Document (1), by referring to the conformation ($Ca^{2+}$) specificity of the anti-HPC monoclonal antibody, indicated to the skilled person that calcium ions influenced the conformation of HPC, and thus its binding to and elution from ligands.

(ii) The document described how, prior to the key step of passage through an immunoaffinity column, HPC was pre-purified by a procedure including: (1) treatment with an EDTA-containing buffer, (2) contact with an anion-exchange column so as to promote binding and (3) elution with a buffer containing 10mM $CaCl_2$. In this procedure, the skilled person would have readily

recognised, based on his general knowledge and on the information provided by document (1) itself, the chelating function of EDTA and the conformational effect of the calcium ions.

(iii) Although no data about the degree of purification achieved in the said preliminary steps were reported in the document, the skilled person was informed that the recovery was quite high **at each step** and that the recovered product was fully functional **through each step** (cf the relevant passages of the document quoted in point 10 above).

(iv) The skilled person, although recognising that the key step of the procedure described in document (1) was the passage through the immunoaffinity column, would have seen that the said step was carried out **in order to achieve homogeneity**, but would not have doubted that some degree of purification was already achieved by the preliminary procedure.

(v) Thus, when devising an alternative method for purifying HPC, the skilled person would have readily taken into consideration the option of a method **comprising** the pre-purification procedure described in document (1), which corresponds in fact to steps (a) to (c) of the method of claim 1, because it was a known, simple and safe way of operating. The subsequent removal of the calcium ions with a chelating agent, eg EDTA (cf step (d) of claim 1) was also quite straightforward for the skilled person in view of the knowledge that, as the said ions had a conformational effect on the molecule, they had to be removed or added depending on the technical circumstances: for

example, a further passage on an anion-exchange resin, would have required their removal with a chelating agent as done already in the first step of the pre-purification procedure (cf item ii)1) above).

15. In the board's judgement, no inventive contribution to art can be seen in proposing in general terms a purification method which is characterised by the fact that it **comprises** a known sequence of purification steps in combination with a trivial measure. In this respect, the appellant's argument that the authors of document (1) had not recognised that a sufficient purification was achieved already before immunoaffinity chromatography and thus one could dispense with using the latter step is not convincing because, firstly, the method of claim 1 does not set any specific level of purity which should be achieved, nor - as already noted - it excludes carrying out in addition to steps (a) to (d) additional purification steps (the use of a second ion-exchange resin is in fact specifically referred to in the description; cf page 4, line 46).

16. For these reasons, claim 1 is found to lack an inventive step, and consequently the request of which it is part, is not allowable under Article 56 EPC.

*Other matters*

17. As the opponents-respondents have not lodged an appeal against the decision of the opposition division, the maintenance of the patent as amended cannot be challenged (cf G 9/92 OJ EPO 1994, 875).

**Order**

**For these reasons it is decided that:**

The appeal is dismissed.

The Registrar: The Chairperson:

U. Bultmann U. Kinkeldey