

Internal distribution code:

- (A) [-] Publication in OJ
- (B) [-] To Chairmen and Members
- (C) [-] To Chairmen
- (D) [X] No distribution

**Datasheet for the decision
of 16 October 2020**

Case Number: T 0533/17 - 3.3.05

Application Number: 06718965.4

Publication Number: 1871503

IPC: B01D15/30, B01D15/32, G01N30/46

Language of the proceedings: EN

Title of invention:
METHODS FOR SEPARATING COMPOUNDS

Patent Proprietor:
Waters Technologies Corporation

Opponent:
Agilent Technologies, Inc.

Headword:
Methods for separating compounds/Waters Technologies

Relevant legal provisions:
EPC Art. 54(1), 54(2), 56, 84, 83, 123(2)

Keyword:

Novelty - auxiliary request (yes)

Inventive step - non-obvious alternative - ex post facto
analysis - auxiliary request (yes)

Sufficiency of disclosure - (yes)

Amendments - allowable (yes)

Decisions cited:

G 0003/14

Catchword:



Beschwerdekammern
Boards of Appeal
Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 0533/17 - 3.3.05

D E C I S I O N
of Technical Board of Appeal 3.3.05
of 16 October 2020

Appellant: Agilent Technologies, Inc.
(Opponent) 5301 Stevens Creek Boulevard
Santa Clara, CA 95056 (US)

Representative: Dilg, Haeusler, Schindelmann
Patentanwalts-gesellschaft mbH
Leonrodstraße 58
80636 München (DE)

Respondent: Waters Technologies Corporation
(Patent Proprietor) 34 Maple Street
Milford, MA 01757 (US)

Representative: Forresters IP LLP
Skygarden
Erika-Mann-Straße 11
80636 München (DE)

Decision under appeal: **Interlocutory decision of the Opposition
Division of the European Patent Office posted on
23 December 2016 concerning maintenance of the
European Patent No. 1871503 in amended form.**

Composition of the Board:

Chairman G. Glod
Members: T. Burkhardt
P. Guntz

Summary of Facts and Submissions

- I. The appeal lies from the opposition division's interlocutory decision that, account being taken of the amendments made by the patent proprietor during the opposition proceedings, the patent EP-B-1 871 503 on the basis of the claims of the main request as filed with a submission dated 24 July 2015 meets the requirements of the EPC.
- II. The following documents were among those discussed during the opposition proceedings:
- D1 H. Lindner *et al.*, J. Chromatogr. A, 782, 1997, 55-62
- D2 M. M. Bushey, J. W. Jorgenson, Anal. Chem., 62, 1990, 161-167
- D3 A. J. Alpert, Journal of Chromatography, 499, 1990, 177-196
- D4 Datasheet "Agilent ZORBAX Bio Series GF-250", 1-4
- D5 E. S. Grumbach *et al.*, LCGC North America, 22, 10, 2004, 1010-1023
- D9 J. Dolan, "A guide to HPLC and LC-MS Buffer Selection", 1-16
- D12 US 5,658,739 A
- D13 H. Tanaka *et al.*, J. Chromatogr. A, 987, 2003, 119-125
- D14 C. A. Mizzen, PhD thesis, University of Toronto, Department of Physiology, 2001, pages ii to xiv and 1 to 230

III. With their statement setting out the grounds of appeal, the appellant (opponent) further submitted the following documents:

- D15 M. T. W. Hearn, "Separation Science and Technology", Volume 2, Chapter 3, Academic Press, 2000, 71-235
- D16 J. R. Litowski *et al.*, J. Peptide Res., 54, 1999, 1-11
- D17 A. P. McKeown *et al.*, J. Sep. Sci., 24, 2001, 835-842
- D18 E. Van Gyseghem *et al.*, J. Chromatogr. A, 988, 2003, 77-93

IV. With a submission dated 6 August 2018, the appellant further submitted the following documents:

- D19 P. Rainville *et al.*, ASMS, Orlando, Florida, 3 June 2002, Poster 115, print-out
- D19a Abstract of D19
- D19b Screenshots from the ASMS 2002 webpage

V. The appellant requested that the decision under appeal be set aside and that the European patent be revoked.

The respondent (patent proprietor) requested that the appeal be dismissed or, in the alternative, that the patent be maintained in amended form on the basis of either the first or the second auxiliary request, as submitted with the letter dated 18 October 2016.

VI. Claims 2 and 20 of the main request, which was upheld by the opposition division, read as follows:

"2. A method for separating a plurality of analytes in a sample, the method comprising:

a) subjecting at least a portion of the sample to a first liquid chromatographic separation mode at a first pH;

b) collecting at least one fraction from the first chromatographic separation; and

c) subjecting the at least one fraction to a second liquid chromatographic separation mode at a second pH; under conditions such that at least two analytes in the sample are separated;

wherein the first pH and the second pH are different and wherein the first and second liquid chromatographic separation modes are hydrophilic interaction chromatography (HILIC)."

"20. A method for purifying a compound in a sample containing the compound and at least two impurities, the method comprising:

a) subjecting the sample to a first liquid chromatographic separation mode at a first pH, under conditions such that the compound is separated from a first impurity;

b) collecting at least one compound-containing fraction from the first chromatographic separation;

c) subjecting the at least one compound-containing fraction to a second liquid chromatographic separation mode at a second pH, under conditions such that the compound is separated from a second impurity; and

d) collecting the purified compound;

wherein the first pH and the second pH are different and wherein the first and second liquid chromatographic separation modes are hydrophilic interaction chromatography (HILIC)."

VII. Independent claims 1 and 17 of auxiliary request 1 read as follows:

"1. A method for analyzing a sample containing at least one analyte, the method comprising:

- a) subjecting the sample to a first liquid chromatographic separation mode at a first pH with a first mobile phase;
- b) collecting at least one fraction from the first chromatographic separation;
- c) subjecting the at least one fraction to a second liquid chromatographic separation mode at a second pH with a second mobile phase; and
- d) detecting the presence or absence of the at least one analyte in the sample;

wherein the first pH and the second pH are different and wherein the first and second liquid chromatographic separation modes are hydrophilic interaction chromatography (HILIC)."

"17. A method for characterizing a sample containing a plurality of polypeptides in a multi-dimensional liquid chromatography system, the method comprising:

- a) injecting the sample into a first dimension chromatography apparatus of said multi-dimensional liquid chromatography system;
- b) chromatographically separating at least a first polypeptide component of said sample from at least a second polypeptide of said sample in a chromatography column of said first dimension chromatography apparatus using a first mobile phase;
- c) eluting said separated first and second polypeptide components in an eluent from said chromatography column;
- d) sampling at least one discrete volume of said eluent;
- e) injecting said at least one discrete volume into a second dimension chromatography apparatus of said multi-dimensional liquid chromatography system;

f) subjecting the injected discrete volume to a chromatographic separation in a chromatography column of said second dimension chromatography apparatus using a second mobile phase, wherein the pH of said first and second mobile phases differs by about 3 pH units;
g) characterizing an eluent from said chromatography column of said second dimension chromatography apparatus using mass spectroscopy, thereby characterizing the sample containing a plurality of polypeptides;
wherein the mode of the first and second dimension chromatography apparatus is hydrophilic interaction chromatography (HILIC)."

Claims 2 to 16 directly or indirectly depend on claim 1, while claim 18 depends on claim 17.

VIII. The arguments by the appellant, where relevant to the present decision, are summarised as follows:

The documents D15 *et seqq.* should be considered.

Claims 2 and 20 of the main request did not fulfil the requirements of Article 123(2) EPC because of the feature "wherein the first and the second pH are different".

The feature "the first and second chromatographic separations are substantially orthogonal to each other" in claim 3 of the main request was a "result to be achieved" and furthermore did not fulfil the requirements of Article 83 EPC.

The term "hydrophilic interaction chromatography (HILIC)" merely required the presence of "hydrophilic interaction". Therefore, the subject-matter of claim 1

of the main request was not novel over D2 (Article 54(1) and (2) EPC).

The claimed subject-matter was moreover obvious in view of each of D1, D3, D15 (common general knowledge) and D19.

IX. The arguments by the respondent, where relevant to the present decision, are summarised as follows:

While D15 might be considered to provide evidence of the common general knowledge, the new inventive-step objection based on this document should not be considered.

The documents D16 *et seqq.* should not be considered in the appeal proceedings.

The appellant's arguments were rebutted. The main request and both auxiliary requests fulfilled the requirements of the EPC.

Reasons for the Decision

1. Consideration of documents
 - 1.1 The two parties concur that **D15** provides evidence of the common general knowledge of the skilled person, and that D15 may be considered in this regard.
 - 1.2 Documents D16 to D18 are said to have been submitted in response to "deficiencies of the opposition division in understanding of what are routine measures in the technical field" which "have only become apparent in the course of the oral proceedings", which resulted in

the opposition division acknowledging an inventive step in the main request (letter of 10 January 2018, page 1, last paragraph).

These documents allegedly show that it is a routine measure to vary the pH in high performance liquid chromatography (HPLC) or HILIC.

However, in their reply to the notice of opposition dated 24 July 2015 (page 6, paragraph 1, page 7 paragraph 2) the respondent has already stated that none of the documents D1 to D11 discloses a method with two chromatographic modes of the same type (let alone of the HILIC type) and with different pH.

Moreover, D16 to D18 are scientific papers and cannot be considered as evidence of the common general knowledge. These documents should already have been filed at the opposition stage. Therefore, these documents are not considered (Articles 12(4) RPBA 2007 and 25(2) RPBA 2020).

- 1.3 In the appellant's view, the poster D19 and the related documents D19a and D19b should be considered since the respondent "apparently decided to conceal this earlier own publication". Such behaviour should not be rewarded by not admitting these documents.

However, it is noted, first, that Rule 42(1)(b) EPC does not put a stringent obligation on the applicant to acknowledge prior art known to him already at the time of filing the application. Therefore, the failure to acknowledge D19 in the patent in suit is not a sufficient reason for admitting this document into the appeal proceedings.

Secondly, the appellant has not provided a convincing justification of why it was possible to find D19 when preparing for the oral proceedings relating to the patent originating from the divisional European patent application No. 12 004 053, but not when preparing the present case at the opposition stage. In the present case, the amendments to the main request were made as long ago as 2015, and auxiliary request 1 differs therefrom only in the deletion of claims. Consequently, the amendments cannot be considered to have triggered an additional search late in the appeal stage.

For these reasons, the documents D19, D19a and D19b should already have been filed at the opposition stage, and are not considered, in accordance with Articles 13(1) and 25(3) RPBA 2020.

Main request

2. Amendments

2.1 It has not been contested that claims 2 and 20 are based on original claims 21 and 40 respectively as originally filed. However, these claims do not disclose that the first and second pH values are different.

The paragraph bridging pages 10 and 11 of the application as originally filed discloses embodiments with different first and second pH, but only in combination with the fact that this difference is *at least 3 pH units* (page 10, line 31).

2.2 The preceding passage on page 10, lines 26 to 28, of the application as originally filed only discloses different pH *ranges*, not a different pH *per se*. The

omission of the word range is an unallowable generalisation.

The wording of:

- page 2, lines 23 to 25 ("varying the pH of the mobile phase used in the two separations") in combination with page 3, lines 1 to 18 (HILIC), and
- page 17, lines 17 to 21 ("changes in pH of the mobile phase")

of the application as originally filed does not only encompass the possibility of using mutually different pH in the two chromatographic dimensions but also the possibility of varying the same pH in both dimensions simultaneously over time, i.e. to have the same pH gradient in each dimension.

Thus, the feature "wherein the first and the second pH are different" in claims 2 and 20 is not directly and unambiguously disclosed in the application as filed.

Consequently, claims 2 and 20 of the main request do not meet the requirements of Article 123(2) EPC.

Auxiliary request 1

3. Amendments

In auxiliary request 1, claims 2 and 20 from the main request have been deleted.

No objections to this request have been raised under Article 123(2), and the board has no objections either.

4. Clarity and sufficiency of disclosure

In the appellant's view, the feature "the first and second chromatographic separations are substantially orthogonal to each other" in dependent claim 2 is "nothing more than a result to be achieved ... and is furthermore objectionable under Article 83 EPC" (statement setting out the grounds of appeal, page 25, penultimate paragraph).

The appellant's objection relates, if anything, to Article 84 EPC. However, the subject-matter of this claim is already present in the patent as granted (as dependent claim 3) and it is not apparent that non-compliance with Article 84 EPC has been introduced by the amendments made to claim 1 (see G 3/14, catchword).

Consequently, the requirements of Article 83 EPC are met, and this issue is not open to debate under Article 84 EPC.

5. Claim interpretation

The appellant argues that the feature "hydrophilic interaction chromatography (HILIC)" in the independent claims merely requires the presence of "hydrophilic interaction, i.e. ionic interactions, hydrogen bondings and/or dipole-dipole interactions" (statement setting out the grounds of appeal, page 9, paragraphs 2 and 3). Correspondingly, paragraph [0047] of the patent in suit would merely require a specific type of column to be used in HILIC. The mobile phase, on the other hand, was not restricted.

This argument is not convincing. The term HILIC is known by the skilled person, e.g. from D3 (title), D5 (title) or D15 (e.g. page 75, lines 8 to 16).

Accordingly, HILIC implies the use of a hydrophilic stationary phase (such as silica) *and* of a hydrophobic mobile phase (such as a mostly organic solvent, e.g. acetonitrile).

This is explained in D3 (summary, page 178, paragraph 2, page 179, paragraph 5) or D5 (page 1012, left-hand column, first full paragraph). The cited passage of D5 moreover indicates that D3 represents the origin of the HILIC technique.

Consequently, the board sees no reason to deviate from the opposition division's view (Reasons 4.2).

6. Novelty

6.1 The appellant submits that the subject-matter of independent claim 1, which is identical to claim 1 of the main request, is anticipated by D2 (Article 54(1) and (2) EPC).

6.2 D2 discloses a first chromatographic dimension with a cation exchange column of Aquapore CX-300 and a second chromatographic dimension with a gel filtration column of Zorbax BioSeries GF-250 (page 163, left-hand column, last paragraph).

6.3 However, since the mobile phase in D2, i.e. *aqueous* NaH₂PO₄ (+Na₂SO₄) (page 163, right-hand column, penultimate paragraph), is not hydrophobic, the dimensions of D2 are not operated in HILIC mode (see point 5.).

6.4 The appellant's argument that the stationary phases of D2 are *suitable* for operation in HILIC mode, as allegedly shown by D14 and D4, is not sufficient, since claim 1 is a method claim; so the corresponding method has to be conducted in HILIC mode (that is, with a hydrophobic mobile phase) for it to be prejudicial to novelty.

6.5 Hence, the subject-matter of claim 1 is novel over D2 (Article 54(1) and (2) EPC).

6.6 Since independent claim 17 and the dependent claims also specify that the two dimensions are HILIC, the subject-matter of these claims is also novel over D2 (Article 54(1) and (2) EPC).

7. Inventive step

The appellant attacked the subject-matter of the claims in relation to each of D1, D3, D15 and D19.

As set out below, the subject-matter of the claims is inventive within the meaning of Article 56 EPC.

The invention relates to liquid chromatographic methods for analysing and characterising a sample.

7.1 The appellant considers the common general knowledge as illustrated by the textbook D15 to be the closest prior art.

The question of the admissibility of this inventive-step objection does not need to be dealt with, since the objection fails of itself, for the following reasons.

D15 deals with the purification and analysis of polypeptides by high performance liquid chromatographic techniques (HPLC) (title).

On page 75 (lines 8 to 16), different high performance liquid chromatographic modes are disclosed, such as reversed-phase (RPC), hydrophilic interaction (HILIC) or "mixed mode combinations".

The passage on page 169, line 44, to page 170, line 29, discloses various aspects and variants of HPLC separation of polypeptides and proteins:

- the application of "different ... pH value" in the "different HPLC modes" of "multidimensional separation strategies" (page 170, lines 20 and 21) carried out in
 - "tandem columns packed with the same or different sorbents" (line 22)
- the "positive-negative" adsorption mode to "ensure that the components of interest are either adsorbed very strongly, or alternatively not absorbed" (lines 27 and 28)

As D15 has the same purpose as the patent in suit and has several features in common with the claimed subject-matter, D15 is a suitable starting point for assessing inventive step.

7.1.1 Problem to be solved

According to the patent in suit, the problem to be solved is the provision of chromatographic methods that allow for the rapid identification of components in a complex mixture of components (paragraph [0027]).

7.1.2 Proposed solution

The patent in suit proposes to solve this problem by the method according to claim 1, characterised at least in that:

- both the first and the second chromatographic separation modes are HILIC, and
- the pH of the two modes are different.

7.1.3 Success of the solution

As indicated in the patent in suit (paragraph [0059]), none of the examples falls under the scope of the invention. Indeed, none of these examples discloses two successive HILIC modes.

Consequently, no effect relating to the use of two HILIC modes with different pH has been shown.

7.1.4 Reformulation of the technical problem

Hence, the technical problem to be solved has to be reformulated in a less ambitious manner, and can be seen as the provision of an *alternative* method for analysing a sample.

This formulation of the problem has been acknowledged by both parties.

7.1.5 Obviousness

As indicated above (see point 7.1), D15 discloses various features of claim 1 at different locations.

This has not been disputed.

However, the passage on page 174, last line, to page 175, line 32, discloses some general "rules" for the fractionation of biopolymers by HPLC, and in particular to "[e]mploy sequential separation processes based on different physical, chemical, or biological properties that are synergistic, and thus *orthogonal, rather than repetitive*" (emphasis added by the board) (page 175, lines 10 to 13).

Correspondingly, page 170, line 19 contemplates the use of "different ... pH value[s]", but only for "*different HPLC modes*" (emphasis added by the board).

As indicated on page 75, lines 8 to 16, the term "modes" designates the modes HILIC, RPC, etc. Hence, the passage on page 170 covers for instance a case where one column works in the HILIC mode and the other in the RPC mode. By contrast, it does not encompass a case where both chromatographic *modes* are HILIC, and only the HILIC *operating conditions* (such as the pH) differ between the two modes.

Consequently, the application of two HILIC dimensions involving the use of different pH in these dimensions amounts to an *ex post facto* analysis.

For these reasons, the subject-matter of claim 1 is inventive when D15 is considered as closest prior art (Article 56 EPC).

- 7.2 D1, which was considered to be the closest prior art in the impugned decision, is not a better starting point for assessing inventive step.

D1 discloses a chromatographic method with two dimensions, namely reversed-phase chromatography (RPC) followed by HILIC (abstract).

The pH in the second dimension is 3.0 (page 57, right-hand column, paragraph 2). It has not been contested that the pH of the first stage is different, i.e. 2.0, as indicated by D9 (page 6, table 2).

Consequently, the subject-matter of claim 1 differs from D1 in that the first chromatic separation mode is (also) HILIC, and that the two HILIC modes have a different pH.

It was acknowledged by the parties that, in this case too, the problem to be solved can be seen as the provision of an *alternative* method for analysing a sample.

7.3 The solution to the problem is not obvious for the following reasons:

In order to arrive at the claimed subject-matter, the skilled person starting from D1 would have to replace the reversed-phase chromatography (RPC) in the first dimension of D1 by HILIC with a different pH from that of the second HILIC dimension.

The appellant argues that the skilled person would replace the first RPC dimension in D1 by HILIC without inventive skill, and that moreover the skilled person would refrain from reproducing the HILIC arrangement of the second dimension in the first dimension of D1 in order to assure a certain degree of orthogonality between the dimensions. This would, in turn, inevitably result in different pH in the two dimensions.

This line of argument is not convincing. It is in fact incomprehensible why the skilled person should first replace RCP by HILIC, thus reducing the degree of orthogonality between the two dimensions (since the second dimension of D1 is already HILIC), only to then choose a different pH in order to increase the degree of orthogonality again.

A combination of D1 with any of D3, D5, D12, D13, D15 does not lead to the claimed subject-matter either:

- D15 - which represents the common general knowledge - instead recommends, as indicated above (see point 7.1.5), the use of *different HPLC modes* in a tandem arrangement in order to increase orthogonality. It is recalled that, according to page 75 of D15, the *modes* in this context are RPC, HILIC etc., and not two different HILIC arrangements.

- None of D3, D5 and D13 discloses a two-stage process, let alone a process with two HILIC stages.

- D12 discloses two RPC modes (column 17, line 61, to column 18, line 46). HILIC is not disclosed. Consequently, when contemplating D12, the skilled person would not arrive at a sequence of two HILIC modes.

- As indicated above (point 1.2), documents D16 to D18 are not considered in the appeal proceedings.

7.4 D3 is more remote from the claimed subject-matter, since it relates to one-dimensional chromatography, whereas D15 and D1 relate to two-dimensional chromatography.

Consequently, D3 is less suitable as the closest prior art than D15 and D1.

7.5 D19 is not considered in the proceedings, as explained above.

7.6 Consequently, the subject-matter of claim 1 involves an inventive step.

7.7 Since independent claim 17 and dependent claims 2 to 16 and 18 also specify that the two dimensions are HILIC with a different pH, the subject-matter of these claims is inventive for the same reasons (Article 56 EPC).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.
2. The case is remitted to the opposition division with the order to maintain the patent in amended form on the basis of auxiliary request 1 filed on 18 October 2016 and a description to be adapted accordingly.

The Registrar:

The Chairman:



C. Vodz

G. Glod

Decision electronically authenticated