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**Datasheet for the decision
of 9 February 2022**

Case Number: T 1738/18 - 3.3.01
Application Number: 07828875.0
Publication Number: 2063265
IPC: H01J49/16, H01J49/04,
H01J49/00, G01N27/62, G01N27/64
Language of the proceedings: EN

Title of invention:

METHOD OF PREPARING SAMPLE FOR MATRIX-ASSISTED LASER
DESORPTION IONIZATION MASS SPECTROMETRY AND MATRIX-ASSISTED
LASER DESORPTION IONIZATION MASS SPECTROMETRY

Applicant:

Shimadzu Corporation

Headword:

Sample preparation for MALDI-MS/SHIMADZU

Relevant legal provisions:

EPC Art. 123(2), 56

Keyword:

Amendments - allowable (yes)
Inventive step - (yes)

Decisions cited:

Catchword:



Beschwerdekammern
Boards of Appeal
Chambres de recours

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Case Number: T 1738/18 - 3.3.01

D E C I S I O N
of Technical Board of Appeal 3.3.01
of 9 February 2022

Appellant: Shimadzu Corporation
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Representative: Kilian Kilian & Partner
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Decision under appeal: **Decision of the Examining Division of the
European Patent Office posted on 14 February
2018 refusing European patent application No.
07828875.0 pursuant to Article 97(2) EPC**

Composition of the Board:

Chairman A. Lindner
Members: T. Sommerfeld
R. Romandini

Summary of Facts and Submissions

- I. The appeal lies from the decision of the examining division in which European patent application 07828875.0, based on an international application published as WO 2008/038812, was refused under Article 97(2) EPC.
- II. The examining division's decision is based on the set of claims of the main request which was filed by letter of 4 May 2016, and auxiliary requests 1 and 2 filed by letter of 19 December 2017 and at oral proceedings on 19 January 2018, respectively.
- III. The examining division decided that the main request and auxiliary request 1 did not comply with Article 56 EPC, and pursuant to Rule 137(5) and 137(3) EPC did not admit auxiliary request 2 into the proceedings.
- IV. The applicant (hereinafter appellant) lodged an appeal against the examining division's decision, requesting that it be set aside and that a patent be granted in accordance with the main request or auxiliary requests 1 or 2 on file.
- V. A summons to oral proceedings was issued by the board, followed by a communication pursuant to Article 15(1) RPBA, providing the board's preliminary opinion on the case.
- VI. By email dated 8 February 2022, the appellant filed a further auxiliary request.
- VII. Oral proceedings took place on 9 February 2022, as a videoconference with the agreement of the appellant. At

the oral proceedings, the appellant withdrew its pending main request and made the pending auxiliary request 1 its main request, while the auxiliary request filed on 8 February 2022 was made the first auxiliary request. At the end of the oral proceedings the chairman announced the board's decision.

VIII. The **main request** comprises 4 claims, independent claims 1 and 3 of which read as follows:

"1. A method of preparing a sample for matrix-assisted laser desorption/ionization mass spectrometry comprising the steps of:

preparing a solution of 2,5-dihydroxy benzoic acid as a matrix solution, and dispensing the matrix solution from an inkjet mechanism onto the surface of a sample to be analyzed to crystallize the 2,5-dihydroxy benzoic acid,

wherein the sample to be analyzed is a biological specimen held on a surface of an electrically conductive support, and

wherein the biological specimen is a section specimen,

characterized in that the concentration of the solution of 2,5-dihydroxy benzoic acid is 40 mg/mL to saturated, and

in that the matrix solution is dispensed at a dispensing pitch of 150 to 175 μm ."

"3. A matrix-assisted laser desorption/ionization mass spectrometry, comprising the steps of:

preparing a sample for matrix-assisted laser desorption/ionization mass spectrometry by the method according to claim 1, and

measuring the sample by a mass spectrometer."

Claim 2 is dependent on claim 1 and further specifies that the biological specimen is derived from a paraffin-embedded specimen.

Claim 4 is dependent on claim 3 and further specifies that second or higher order of multi-stage MS (mass spectrometry) is conducted.

IX. The documents cited in the examination and appeal proceedings include the following:

D1 S. Shimma et al., J. Mass Spectrom. Soc. Jpn.,
2006, 54(4), 133-140

X. The appellant's arguments, in so far as relevant to the present decision, may be summarised as follows:

The basis for the combination of features of claim 1 could be found on pages 7 to 9 and on page 24, first full paragraph, of the application as filed.

Document D1 was the closest prior art. The claimed subject-matter differed from D1 not only in that the concentration of 2,5-dihydroxy benzoic acid (DHB) was 40 mg/mL to saturated but also in that the dispensing pitch was 150 to 175 μm . This specific dispensing pitch was not disclosed in the prior art, and surprisingly provided exceptionally good analytical results in MALDI-MS, as shown on page 48 and Figure 5 of the application as filed. The technical problem could thus be formulated as the provision of a process that allowed the biological sample to be measured evenly and effectively, this being an improvement over D1. It was true that the effect had been shown in the application when using a specific method of sample preparation, but there would be no reason to doubt that the same effect

could be expected with other methods of sample preparation as well. It would not have been obvious to the skilled person to choose this dispensing pitch in the expectation of obtaining the effect shown in the application.

- XI. The appellant requested that the decision under appeal be set aside and that a patent be granted in accordance with the main request filed as auxiliary request 1 on 19 December 2017 or alternatively in accordance with auxiliary request 1 filed by email of 8 February 2022, or auxiliary request 2 filed by letter dated 19 January 2018.

Reasons for the Decision

1. The appeal is admissible.

Main request

2. Article 123(2) EPC

- 2.1 The originally filed claim 1 read:

"1. A method of preparing a sample for matrix-assisted laser desorption/ionization mass spectrometry comprising the steps of:

preparing a solution of 2,5-dihydroxy benzoic acid of 40 mg/mL to saturated concentration as a matrix solution; and

dispensing the matrix solution to a sample to be analyzed by using an inkjet mechanism, to crystallize the 2,5-dihydroxy benzoic acid."

2.2 Claim 1 of the main request differs from claim 1 as originally filed in that the following features have been added:

- "wherein the sample to be analyzed is a biological specimen held on a surface of an electrically conductive support,"
- "wherein the biological specimen is a section specimen,"
- "and in that the matrix solution is dispensed at a dispensing pitch of 150 to 175 μm ."

2.3 The basis for these features can be found on pages 7 to 9, and 24, first full paragraph of the application as filed. On page 7, lines 10 and 11, it is disclosed that "The present invention includes the following aspects (1) to (6)". This passage is first followed by item (1) which is verbatim identical to claim 1 as originally filed, and then by item (2) which reads: *"The method of preparing a sample for matrix-assisted laser desorption/ionization mass spectrometry according to (1), wherein the sample to be analyzed is a section specimen of a biological sample"*. On page 9, item (4) reads: *"The method of preparing a sample for matrix-assisted laser desorption/ionization mass spectrometry according to any one of (1) to (3), wherein the sample to be analyzed is held on a surface of an electrically conductive support"*. Finally, the passage on page 24, first full paragraph reads: *"In the present invention, it is possible to produce crystals in which the matrix is uniformly deposited at any position on the biological sample, by appropriately setting a dispensing pitch. Such a dispensing pitch is 100 to 200 μm , more preferably 125 to 200 μm , and further preferably 150 to 175 μm "*.

2.4 The board thus comes to the conclusion that the above-mentioned passages provide a basis not only for each of the added features individually but also for their combination in the present claim 1. Hence claim 1 of the main request complies with Article 123(2) EPC.

2.5 The same applies to the remaining claims of the main request. The basis for claim 2 is to be found in item (3) on page 8, which specifies that the sample to be analysed is derived from a paraffin-embedded specimen. Claim 3 finds its basis in item (6) on page 9, line 25 to page 10, line 5, and claim 4 finds its basis in item (7) on page 10.

2.6 The claims of the main request thus comply with Article 123(2) EPC.

3. Article 56 EPC

3.1 The present application relates to a method of preparing a sample for matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, in particular for a section specimen of a biological sample, and more particularly to a mass spectrometric imaging method for a section specimen of a biological sample (page 1, lines 13 to 21 of the application as filed). For the purposes of MALDI mass spectrometry, the production of a uniform crystal using a matrix is an important factor determining the likelihood of ionization of the molecule to be measured, which greatly contributes to sensitivity, quantitative performance and reproducibility of analysis, these being properties which are particularly relevant for automatic analysis (page 2, lines 5 to 11).

- 3.2 Document D1, which also discloses a method of preparing a sample for MALDI mass spectrometry, is the closest prior art. The subject-matter of claim 1 differs from the disclosure of D1 in that a concentration of 2,5-dihydroxy benzoic acid (DHB) of 40 mg/mL to saturated is used and in that the matrix solution is dispensed at a dispensing pitch of 150 to 175 μm . The technical effect associated with these differences is that exceptionally good analytical results are achieved in MALDI mass spectrometry, as evidenced by Example 5 (page 48, lines 5 to 15 and Figure 5). The objective technical problem can thus be formulated as the provision of an improved process for sample preparation which allows even and effective ionization of the biological molecule to be measured (page 24, last paragraph). In view of the results disclosed on page 48 and shown in Figure 5, the board is satisfied that the claimed solution solves the technical problem.
- 3.3 While it might have been routine for the skilled person to determine appropriate DHB concentrations and dispensing pitches for MALDI mass spectrometry, there is no teaching or suggestion in the prior art pointing to a method making use of DHB in the claimed concentration together with a dispensing pitch of 150 to 175 μm in order to arrive at a process with the advantages as described above. Moreover, although the claim is not restricted to the method which has been used in Example 5, namely a method using paraffin sections subjected to a given method for deparaffinization, the board considers that the same advantageous results reported in Example 5 and shown in Figure 5 could also be expected for biological section specimens which have been deparaffinized by a different process, as long as the other method conditions, namely the DHB concentration and the dispensing pitch, are the

same. In this context, the board disagrees with the conclusions of the examining division that the problem could not be considered solved over the whole scope because other experimental parameters (such as the number of droplets per spot and the size of individual droplets) would have an impact on the optimum dispensing pitch. The board fails to see any evidence on file for these conclusions.

- 3.4 The board therefore comes to the conclusion that the subject-matter of claim 1 of the main request involves an inventive step. The same conclusion applies to independent claim 3, which is directed to MALDI mass spectrometry comprising the steps of preparing a sample according to claim 1 and measuring it by a mass spectrometer, and to the dependent claims 2 and 4. Hence the main request complies with Article 56 EPC.
4. No further objections were raised by the examining division. The board has no further objections either. Hence the main request is considered to comply with the requirements of the EPC.

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 with the order to grant a patent on the basis of the following claims and a description and drawings possibly to be adapted to:

Claims 1 to 4 of the main request, filed as auxiliary request 1 of 19 December 2017.

The Registrar:

The Chairman:



M. Schalow

A. Lindner

Decision electronically authenticated