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Datasheet for the decision of 29 August 2019

Case Number: T 1131/15 - 3.3.01

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Language of the proceedings: EN

Title of invention:
DETECTING SUCCINYLACETONE

Patent Proprietor:
PerkinElmer Health Sciences, Inc.
Azienda Ospedaliero Universitaria Meyer di Firenze

Opponent:
Recipe Chemicals + Instruments GmbH

Headword:
Succinylacetone/PERKIN

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
Novelty - (yes)
Inventive step - (yes)

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Case Number: T 1131/15 - 3.3.01

D E C I S I O N
of Technical Board of Appeal 3.3.01
of 29 August 2019

Appellant: (Opponent)	Recipe Chemicals + Instruments GmbH Dessauerstr. 3 80992 München (DE)
Representative:	Rupp, Christian Mitscherlich PartmbB Patent- und Rechtsanwälte Sonnenstraße 33 80331 München (DE)
Respondents: (Patent Proprietors)	PerkinElmer Health Sciences, Inc. 940 Winter Street Waltham, MA 02451 (US)
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Representative:	Vossius & Partner Patentanwälte Rechtsanwälte mbB Siebertstrasse 3 81675 München (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 2 April 2015 concerning maintenance of the European Patent No. 2155185 in amended form**

Composition of the Board:

Chairman	A. Lindner
Members:	J. Molina de Alba
	M. Blasi

Summary of Facts and Submissions

I. The opponent (appellant) filed an appeal against the interlocutory decision of the opposition division that European patent No. 2 155 185 in the version of the main request pending before it, and the invention to which it relates, met the requirements of the EPC. The main request contained two independent claims, namely claims 1 and 13. Claim 1 reads as follows:

"*1. A method for detecting a biologically active ketone, the method comprising:*

contacting a sample with an extraction solution comprising a C1-3 linear or branched chain monoalcohol and a strong base;
derivatizing a biologically active ketone in the sample; and
evaluating the derivatized biologically active ketone in the derivatized sample using tandem mass spectrometry, wherein the biologically active ketone is succinylacetone or a steroid."

In the following, succinylacetone will be referred to as "SA".

II. The evidence cited by the parties during the opposition/appeal proceedings include the following prior art documents:

D1: J. Sander et al., Clinical Chemistry, 52(3), 2006, 482-487

D4: P. Allard et al., Clinical Biochemistry, 37, 2004, 1010-1015

III. In the decision under appeal, the opposition division considered that the amended patent did not add subject-matter and that the claimed methods were sufficiently disclosed, novel and inventive.

IV. In the statement of grounds of appeal, the appellant contested the opposition division's view in relation to novelty and inventive step. On the issue of sufficiency of disclosure, it referred to the arguments presented in the notice of opposition.

V. In their reply to the statement of grounds of appeal, the respondents (patent proprietors) requested that the appeal be dismissed. Alternatively, they requested that the patent be maintained in amended form on the basis of any of the sets of claims filed on 16 August 2013 as auxiliary requests 1 to 6.

In response to the board's communication in preparation for the oral proceedings scheduled according to the requests of the parties, the respondents filed two additional sets of claims as auxiliary requests 7 and 8.

VI. Oral proceedings were held before the board on 29 August 2019, at which the respondents filed a set of claims of a new main request to replace the previous one.

The set of claims of this new main request consists of claims 1 to 12 of the set of claims which was considered allowable by the opposition division (i.e. claims 13 to 17 have been deleted).

VII. The appellant's arguments, where relevant to the present decision, may be summarised as follows:

The method of claim 1 of the main request is anticipated by the embodiment in document D1 referred to as "direct preparation" (see the passage bridging the columns on page 483). That preparation combines the two steps of the primary method of document D1 into a single step, so that its extraction solution is a combination of methanol (first step) with acetonitrile/ water and hydrazine (second step).

If despite this the method of claim 1 is regarded as novel, then it lacks an inventive step starting from either the direct preparation or the primary, two-step method of document D1.

Starting from the direct preparation, the method of claim 1 would differ in the nature of the solvent in the extraction solution (methanol vs acetonitrile/ water). However, this difference does not result in any effect since, as in the method of claim 1, the extraction solution of the direct preparation is believed to extract not only SA but also amino acids, free carnitine and acylcarnitines. Hence, the technical problem to be solved is the provision of an alternative method for the extraction of SA along with amino acids, free carnitine and acylcarnitines.

As it was generally known that methanol did not impair the reaction of derivatisation of SA with hydrazine, and it was known from the first step of the primary method of document D1 that methanol was suitable for extracting amino acids and acylcarnitines, it was obvious to the skilled person that the combination of the two extraction solutions of the primary method of

document D1 into a single one would be a suitable solution to the problem posed. By implementing that combination, the skilled person would have arrived at a method as disclosed in claim 1, i.e. involving an extraction solution that contains methanol (C1-3 monoalcohol), acetonitrile, water and hydrazine (strong base).

Starting from the two-step method of document D1, the method of claim 1 also differs in that the extraction solution contains a C1-3 monoalcohol and a strong base. As claim 1 does not require the extraction and derivatisation to occur in a single step (the strong base is not necessarily the derivatisation agent), the problem cannot be formulated as the provision of a simpler method but rather as an alternative method for the extraction of SA along with amino acids, free carnitine and acylcarnitines.

For the reasons already explained in relation to the direct preparation, the skilled person would have also combined the two extraction solutions of the primary method of D1 in order to solve the problem posed.

These conclusions are supported by document D4, which teaches on page 1012 (left-hand column, last paragraph) that SA may be extracted either directly from dried blood spots or from residual blood spots (i.e. blood spots that have been previously extracted with absolute methanol).

VIII. The respondents' arguments, where relevant to the present decision, may be summarised as follows:

The method of claim 1 of the main request is novel over the content of document D1 because neither the direct

preparation nor the two-step method of D1 involved the use of a solution containing both a C1-3 alcohol and a strong base. The appellant's allegation that the extraction solution of the direct preparation was a combination of the solutions used in the two-step method is unfounded. The composition of the extraction solution used in the direct preparation is not specified and, from its context, it should be the same as the solution used for extracting the residual dried blood spots, i.e. acetonitrile/water containing hydrazine.

With regard to the question of inventive step, the direct preparation of document D1 cannot represent the closest prior art because it treats the sample with acetonitrile/water and hydrazine, which is a solution suitable for extracting SA but not amino acids, free carnitine and acylcarnitines. The closest prior art is rather the primary method of document D1, where SA, amino acids and acylcarnitines are extracted in two separate steps.

The method of claim 1 requires that the sample be contacted with an extraction solution comprising a C1-3 monoalcohol and a strong base, while the primary method of document D1 comprises the treatment of the sample with two separate extraction solutions consecutively: first absolute methanol and then an acetonitrile/water mixture containing hydrazine, where methanol is evaporated before the sample is treated with acetonitrile/water and hydrazine.

This distinguishing feature of claim 1 allows the extraction of SA along with amino acids, free carnitine and acylcarnitines in a single step, simplifying the primary method of document D1 and saving time and

costs. Accordingly, the technical problem solved by the method of claim 1 is the provision of an improved method for the extraction of SA and other analytes, such as amino acids, free carnitine and acylcarnitines, from a sample.

The method of claim 1 solves the problem posed, as shown in examples 1 to 3 of the patent. In addition, it does it in an inventive manner because there is no indication in the prior art that SA and other analytes could be extracted simultaneously from a sample using an extraction solution comprising a C1-3 monoalcohol and a strong base. Contrary to the appellant's opinion, the skilled person would have no reason to combine the two separate extraction solutions of the primary method of document D1: firstly, the combination was not suggested and, secondly, the skilled person had no reasonable expectation of success that the combination of acetonitrile/water with methanol would successfully extract SA along with other analytes. In this context, the appellant's argument that the skilled person knew that methanol does not negatively affect the action of hydrazine is an unsubstantiated allegation.

The teaching of document D4 does not render the claimed method obvious either, since its content is essentially the same as that of document D1; it teaches the extraction and quantification of SA and further analytes for use in newborn screening programs in two separate steps and does not suggest the combination of methanol with the extraction solution containing acetonitrile/water and hydrazine.

In relation to the issue of sufficiency of disclosure, the appellant merely made reference to its submissions in the notice of opposition and failed to explain the

reasons why the appealed decision was wrong. Thus, the objection of sufficiency of disclosure has not been substantiated.

IX. The final requests of the parties were as follows:

- The appellant requested that the decision under appeal be set aside and the patent be revoked. In addition, the appellant requested that the sets of claims filed by the respondents with the letter dated 29 July 2019 as auxiliary requests 7 and 8 not be admitted into the proceedings.

- The respondents requested that the decision under appeal be set aside and the patent be maintained in amended form on the basis of claims 1 to 12 of the main request filed at the oral proceedings before the board, or alternatively, on the basis of any of the sets of claims filed with the letter dated 16 August 2013 as auxiliary requests 1 to 6, or on the basis of any of the sets of claims filed with the letter dated 29 July 2019 as auxiliary requests 7 and 8.

X. At the end of the oral proceedings, the board's decision was announced.

Reasons for the Decision

1. The appeal is admissible. It complies with the requirements pursuant to Articles 106 to 108 and Rule 99(2) EPC.

2. Teaching of document D1

Document D1 relates primarily to (see abstract and heading "Material and Methods") a method of extraction and quantification of SA in routine newborn screening using tandem mass spectrometry.

This method is based on the observation that, when dried blood spots are extracted with absolute methanol for acylcarnitine and amino acids quantification, SA remains unextracted because it is bound in the form of a conjugate with amino acid residues present in the sample. However, SA may be derivatised and extracted with an acetonitrile/water solution containing hydrazine: the reaction with hydrazine releases SA from the conjugate, which can then be extracted in derivatised form as 3-(5-methyl-1H-pyrazol-3-yl) propionic acid (MPP) (see D1, page 482, right-hand column, last sentence and figure 1). Document D4, which is cited in document D1, discloses essentially the same teaching (see D4, page 1013, right-hand column, second full paragraph).

Thus, document D1 proposes the extraction of "residual" dried blood spots (i.e. dried blood spots that have been extracted with methanol) with an acetonitrile/ water solution containing hydrazine for the extraction and quantification of SA.

For comparative purposes, the authors of D1 also carried out a "direct preparation", where SA was derivatised and extracted directly from dried blood spots which had not been previously extracted with methanol (see paragraph bridging the columns of page 483).

The two methods provided the same quantitative results, although the method which extracted SA from residual dried blood spots had a lower analytical background and was therefore more sensitive (see also D4, page 1012, left-hand column, last paragraph).

3. Novelty - claim 1 of the main request

The appellant argued that the direct preparation in document D1 anticipated the method of claim 1 because it was a combination of the two steps of the primary method (i.e. extraction of dried blood spots with methanol followed by the treatment of the residual blood spots with acetonitrile/water and hydrazine). Thus, the extraction solution used in the direct preparation was a combination of methanol with acetonitrile/water and hydrazine.

The board does not accept this argument: firstly, document D1 does not specify the nature of the extraction solution used in the direct preparation, and there is nothing in the document which supports the appellant's allegation that the extraction solution should be a combination of the two separate extraction solutions of the primary method. Secondly, considering that the direct preparation was intended to compare the extraction of SA from dried blood spots and residual dried blood spots, the board agrees with the respondents that the only reasonable interpretation would be that the extraction solution of the direct preparation was the same as that in the second step of the primary method, i.e. a solution containing acetonitrile/water and hydrazine.

The board then concludes that the subject-matter of claim 1 is novel pursuant to Article 54(1), (2) EPC.

4. Inventive step - claim 1 of the main request

4.1 Field of the invention

The patent relates to the detection and quantification of metabolites used in newborn screening for identifying patients affected by diseases such as hereditary tyrosinemia Type I (see paragraphs [0001] and [0002]). In particular, the patent concerns a method for detecting a biologically active ketone selected from SA and a steroid in a sample, where the sample is contacted with an extraction solution comprising a C1-3 monoalcohol and a strong base. This solution extracts the ketone from the sample in derivatised form for its subsequent evaluation with tandem mass spectrometry. In preferred embodiments, the biologically active ketone is SA (marker of tyrosinemia Type I), the C1-3 monoalcohol is methanol (claim 3), and the strong base is hydrazine (claim 4). The derivatised ketone obtained by the reaction of SA with hydrazine is 3-(5-methyl-1H-pyrazol-3-yl) propionic acid (MPP) (claim 9).

In line with the teaching of documents D1 and D4, the patent explains in paragraph [0026] and figure 2, that SA in biological fluids is generally found in the form of a Schiff base conjugated to the side chains of amino acid residues. The strong base (e.g. hydrazine) in the extraction solution of the invention reacts with the Schiff base to release the ketone in a derivatised form (e.g. MPP), which may then be extracted and quantified using tandem mass spectrometry.

4.2 Closest prior art

4.2.1 The parties agreed that the disclosure of document D1 represents the closest prior art. However, while the appellant considered that both the direct preparation and the primary, two-step method were suitable springboards, the respondents denied the suitability of the direct preparation as the closest prior art.

In the primary method, dried blood spots were contacted with methanol for the extraction of amino acids and acylcarnitines and, subsequently, the residual dried blood spots were contacted with an acetonitrile/water solution containing hydrazine for the derivatisation and extraction of SA.

In the direct preparation, SA was derivatised and extracted directly from dried blood spots without the previous separation of amino acids and acylcarnitines. Although document D1 does not explicitly mention the conditions of derivatisation and extraction in the direct preparation, as mentioned in point 3 above, the board agrees with the respondents that it may be assumed that it was an acetonitrile/water solution containing hydrazine.

As both methods have the same aim as the method of claim 1, i.e. detecting a biologically active ketone (SA) in a sample by derivatisation and extraction of the ketone with a solution containing a strong base (hydrazine), and both methods are taught in D1 to provide the same quantitative results (page 483, passage bridging both columns), the board agrees with the appellant that both of them constitute a suitable starting point for the assessment of inventive step.

4.3 Technical problem solved

4.3.1 Starting from the direct preparation, the method of claim 1 differs in the nature of the solvent used for the derivatisation and extraction of SA: in claim 1 it is a C1-3 monoalcohol while in D1 it is acetonitrile/ water.

The parties disputed whether this difference resulted in any effect. The respondents argued that the extraction solution of the method of claim 1 allowed the extraction and quantification of SA along with amino acids, free carnitine and acylcarnitines. The appellant contended that this effect was also achieved with the solution in the direct preparation (acetonitrile/water with hydrazine).

As there is no evidence on file that the derivatisation and extraction of the biologically active ketone using a C1-3 monoalcohol (e.g. methanol) as solvent instead of an acetonitrile/water mixture results in any effect, the board accepts the appellant's formulation of the technical problem as the provision of an alternative method for the detection of SA along with amino acids, free carnitine and acylcarnitines in a sample.

4.3.2 Starting from the primary, two-step method the board accepts the appellant's argument that the method of claim 1 differs only in the presence of an extraction solution which contains a C1-3 monoalcohol and hydrazine. This follows from the consideration of the appellant's argument that the method of claim 1 does not require that the extraction and derivatisation be carried out in a single step, because the strong base

and the derivatising agent are not necessarily the same.

Accordingly, the board also accepts that the technical problem, as for the direct preparation, may be formulated as the provision of an alternative method for the detection of SA along with amino acids, free carnitine and acylcarnitines.

4.4 The examples in the patent, especially example 3, demonstrate that the method of claim 1 solves the problem posed since they show that solutions comprising methanol and hydrazine are suitable for derivatising and extracting SA along with amino acids, free carnitine and acylcarnitines from dried blood spots, in amounts suitable for quantification. This was not disputed between the parties.

4.5 Obviousness

On this issue, the appellant argued that, starting from either of the methods of document D1, the skilled person would have combined the separate extraction solutions of the primary method of document D1 as an obvious alternative. This was for two reasons: firstly, it was generally known that methanol did not interfere in the reaction of derivatisation of SA with hydrazine and, secondly, it was known from the first step of the primary method of document D1 that methanol was suitable for extracting amino acids and acylcarnitines. Thus, by combining methanol (first step) with acetonitrile/water and hydrazine (second step) the skilled person would have expected to derivatise and extract SA along with amino acids, free carnitine and acylcarnitines. By doing so, the skilled person would

have arrived at the method of claim 1 without involving of an inventive step.

The board disagrees.

On the one hand, the prior art does not contain any indication to combine the separate extraction solutions of document D1 in order to simultaneously extract SA, amino acids, free carnitine and acylcarnitines. In addition, it is unlikely that the skilled person would have considered this combination because it was known from D1 that the acetonitrile/water mixture containing hydrazine was suitable for derivatising and extracting SA, but the suitability of methanol for this purpose was uncertain. In this respect, the board notes that the appellant acknowledged during the oral proceedings before it that the skilled person would not have expected methanol alone to be a suitable extracting medium for SA, since it was known from the first step of the primary method of document D1 that methanol did not extract SA (see also D4, page 1012, left-hand column, last paragraph).

Thus, even acknowledging that the skilled person would not have expected methanol to have a negative influence on the derivatisation reaction of SA with hydrazine, they would have expected it to negatively affect the extracting properties of the acetonitrile/water mixture in relation to SA. Hence, the skilled person would not have considered the methanol/acetonitrile/water combination to be a suitable alternative for extracting the derivatised ketone to an extent sufficient for quantification, which is an essential requirement to establish a reliable diagnosis.

For the same reason, the skilled person, starting from the direct preparation, would also not have replaced acetonitrile with methanol.

Accordingly, from the arguments on file, the board cannot conclude that the skilled person would have arrived at the claimed method without the involvement of an inventive step pursuant to Article 56 EPC.

5. Sufficiency of disclosure

On the issue of sufficiency of disclosure pursuant to Article 83 EPC, the appellant merely referred to the notice of opposition (see point 3 of the statement of grounds of appeal).

In the decision under appeal, sufficiency of disclosure was addressed. Therefore, the appellant's subsequent reference to its written submissions made during the opposition proceedings cannot replace the provision of reasons as to why it is requested that the decision under appeal be reversed in this regard. Thus, in relation to the issue of sufficiency of disclosure the requirements of Article 12(2) RPBA have not been met with the consequence that the issue of sufficiency of disclosure did not form part of the present appeal proceedings under Article 12(4) RPBA.

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 with claims 1 to 12 filed at the oral proceedings before the board, and with a description and drawings to be adapted thereto.

The Registrar: The Chairman:

M. Schalow A. Lindner

Decision electronically authenticated