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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

Ex parte BRETT HOLMQUIST, NIGEL J. CLARKE, ANNE CASTON-BALDERRAMA, and RICHARD E. REITZ

Appeal 2019-001084
Application 14/267,014
Technology Center 1700

Before JEFFREY R. ROBERTSON, MONTÉ T. SQUIRE, and MICHAEL G. McMANUS, *Administrative Patent Judges*.

McMANUS, *Administrative Patent Judge*.

DECISION ON APPEAL

Pursuant to 35 U.S.C. § 134(a), Appellant[1] seeks review of the Examiner's decision to reject claims 1–38. We have jurisdiction under 35 U.S.C. § 6(b).

We AFFIRM.

[1] We use the word "Appellant" to refer to "applicant" as defined in 37 C.F.R. § 1.42. Appellant identifies the real party in interest as Quest Diagnostics Investments Incorporated. Appeal Br. 1.

CLAIMED SUBJECT MATTER

The present application generally relates to the detection of vitamin D metabolites and particularly to the detection of dihydroxyvitamin D metabolites by mass spectrometry. Specification (May 1, 2014) ("Spec.") ¶ 2.

There are two forms of vitamin D: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). *Id.* ¶ 3. Vitamin D_2 is derived from fungal and plant sources. *Id.* ¶ 4. Vitamin D_3 is synthesized *de novo* by animals and people. *Id.* ¶ 3.

In the body, vitamin D_2 is metabolized to form "the metabolites 25-hydroxyvitamin D_2($25OHD_2$) and 1,25-dihydroxyvitamin D_3 [sic D_2] ($1,25(OH)_2D_2$)." *Id.* ¶ 4. Vitamin D_3 is taught to be metabolized by hydroxylation to form the intermediate metabolite 25-hydroxyvitamin D_3 (25-hydroxychole calciferol; calcifediol; $25OHD_3$). *Id.* ¶ 3. Calcifediol is the major form of vitamin D_3 in circulation and is converted to 1,25-dihydroxyvitamin D_3 (calcitriol; $1,25(OH)_2D_3$) "which is generally believed to be the metabolite of vitamin D_3 with the highest biological activity." *Id.* The Specification teaches that serum levels of 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2 (total 25-hydroxyvitamin D; "25OHD") in humans are a useful index of vitamin D nutritional status. *Id.* ¶ 5.

The Specification teaches methods for detecting the amount of a dihydroxyvitamin D metabolite in a sample by mass spectrometry. One such method is described as follows:

> (a) immunopurifying one or more dihydroxyvitamin D metabolites from the sample; (b) further purifying the

> immunopurified dihydroxyvitamin D metabolite(s) by HPLC; (c) determining the amount of the vitamin D metabolites obtained from step (b) by tandem mass spectrometry by: (i) generating a precursor ion of the dihydroxyvitamin D metabolite(s); (ii) generating one or more fragment ions of the precursor ion; and (iii) detecting the presence or amount of one or more of the ions generated in step (c) or (d) or both and relating the detected ions to the presence or amount of the dihydroxyvitamin D metabolite(s) in the sample.

Id. ¶ 9. The Specification further teaches that, in an embodiment, a method is taught for determining "the presence or amount of $1\alpha,25(OH)_2D_2$ and $1\alpha,25(OH)_2D_3$ in a single assay." *Id.*

Claim 1 is illustrative of the subject matter on appeal and is reproduced below with certain limitations bolded for emphasis:

> A method for determining an amount of one or more dihydroxyvitamin D metabolites in a biological sample by tandem mass spectrometry; the method comprising:
>
> (i) adding one or more internal standards to the sample;
>
> (ii) purifying the one or more dihydroxyvitamin D metabolites and the one or more internal standards;
>
> (iii) **derivatizing the one or more dihydroxyvitamin D metabolites and the one or more internal standards with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD)**;
>
> (iv) analyzing the amount of the derivatized one or more dihydroxyvitamin D metabolites and the derivatized one or more internal standards in the sample by tandem mass spectrometry, wherein the tandem mass spectrometry comprises electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI).

Appeal Br. 10 (Claims App'x) (emphasis added).

REFERENCES

The Examiner relies upon the following prior art:

Name	Reference	Date
Clarke	US 2006/0228808 A1	Oct. 12, 2006
Singh	US 2006/0094125 A1	May 4, 2006
Weiskopf	Weiskopf et al. "Examination of structurally selective derivatization of vitamin D_3 analogues by electrospray mass spectrometry." *J. Mass Spectrom*. 2001; 36: 71–78	2001

REJECTIONS

The Examiner maintains the following rejections:

1. Claims 1–12 and 14–23 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Clarke in view of Weiskopf. Final Act. 3–4.
2. Claims 13 and 24–38 are rejected under pre-AIA 35 U.S.C. § 103(a) as being unpatentable over Clarke in view of Weiskopf and further in view of Singh. *Id.* at 5–7.

DISCUSSION

Rejection 1. The Examiner rejects claims 1–12 and 14–23 as obvious over Clarke in view of Weiskopf. *Id.* at 3–4. Appellant argues the rejected claims as a group. *See* Appeal Br. 7–9. We select claim 1 as a representative claim and the remaining claims will stand or fall therewith. *See* 37 C.F.R. § 41.37(c)(1)(iv).

The Examiner finds that Clarke teaches each element of claim 1 other than derivatizing the vitamin D metabolites and the internal standards with PTAD. *Id.* at 3. In this regard, Clarke teaches as follows:

> The present invention provides methods for detecting the presence or amount of a vitamin D metabolite in a test sample by mass spectrometry, including tandem mass spect[r]ometry. **Preferably, the methods of the invention do not include derivatizing the sample or the vitamin D metabolites** prior to the mass spectrometry analysis.

Clarke ¶ 7 (emphasis added).

The Examiner finds that Weiskopf teaches a method of examining vitamin D_3 analogues by mass spectrometry where vitamin D is derivatized with PTAD. Final Act. 3. The Examiner further finds that Weiskopf teaches "that PTAD derivatization is advantageous because it provides structurally selective ions for monitoring side chain metabolism in triple quadrupole and quadrupole ion mass spectrometers." *Id.*

Weiskopf is a journal article that examines mass spectrometry of a vitamin D analogue known as EB 1089. Weiskopf, Abstract. Weiskopf teaches to derivatize (react) EB 1089 with PTAD prior to conducting mass spectrometry. *Id.* at 72. Weiskopf explains, in part, the benefit of PTAD derivatization.

> When compared with GC/MS, one drawback of using API-MS/MS techniques for qualitative analysis is the paucity of structurally informative ion fragments. While this proves to be a considerable disadvantage with underivatized vitamin D compounds, PTAD derivatives yield somewhat more information. PTAD derivatives of most 1-hydroxylated vitamin D compounds, including calcitriol, yield a major ion product at *m/z* 314. The ubiquity of the *m/z* 314 fragment among a wide range of analogues and metabolites has allowed its use as a

> diagnostic ion for both parent-scanning and multiple-reaction monitoring.

Id.

The Examiner concludes that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Clarke et al., wherein PTAD is utilized as a derivatization agent in order to provide structurally selective ions for monitoring side chain metabolism with triple quadrupole and quadrupole ion mass spectrometers as taught by Weiskopf et al." Final Act. 3–4.

Appellant asserts that the rejection is in error on several bases. Appeal Br. 7–9. First, Appellant briefly asserts that "[n]one of the cited references discloses that dihydroxyvitamin D metabolites are derivatized with PTAD." *Id.* at 7. This is not persuasive.

Weiskopf teaches that "PTAD derivatives of most 1-hydroxylated vitamin D compounds, including calcitriol, yield a major ion product at *m/z* 314." Weiskopf 72. Calcitrol is dihydroxyvitamin D_3, a vitamin D_3 metabolite. *See* Spec. ¶ 3. Further, Weiskopf teaches the derivatization of EB 1089 with PTAD. EB 1089 is a dihydroxyvitamin D metabolite. *See* Weiskopf, Figure 1(b); Spec. ¶ 13 (regarding the definition of "metabolite"). Accordingly, we do not find this argument to be persuasive.

In view of the foregoing passage from Weiskopf, we are also not persuaded by Appellant's argument (Appeal Br. 7; Reply Br. 2) that one of ordinary skill in the art would have understood the derivatization techniques disclosed therein to be limited to synthetic vitamin D analogues. Rather, the quoted passage in Weiskopf appears to be general in nature. Further, as the Examiner points out, the prior art and the instant Specification are inclusive

of both natural and synthetic analogues of vitamin D. Ans. 3–4 (citing Clarke ¶ 11 and Spec. ¶ 13).

Second, Appellant argues that "the state of the art teaches away from using APCI to quantitate derivatized vitamin D metabolites." Appeal Br. 7. In support, Appellant argues that a 1993 article by Yeung cited in an Information Disclosure Statement (IDS) during prosecution, but not relied upon by the Examiner, teaches that PTAD derivatives of vitamin D_3 are "too polar to be ionized by traditional **EI or chemical ionization** techniques." *Id.*

The ultimate determination of obviousness under § 103 is a question of law based on underlying factual findings. *In re Baxter Int'l, Inc.*, 678 F.3d 1357, 1361 (Fed. Cir. 2012). These underlying factual considerations include secondary considerations of non-obviousness such as commercial success, long-felt but unsolved needs, failure of others, etc. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406, (2007) (citing *Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966)). Evidence of industry skepticism is a question of fact that weighs in favor of non-obviousness. *WBIP, LLC v. Kohler Co.*, 829 F.3d 1317, 1335 (Fed. Cir. 2016). When evidence of secondary considerations is submitted, we begin anew and evaluate the rebuttal evidence along with the evidence upon which the conclusion of obviousness was based. *In re Rinehart*, 531 F.2d 1048, 1052 (CCPA 1976).

In the Answer, the Examiner takes the position that Yeung was published in 1993 and does not reflect the state of the art at the time of application. Answer 4. The Examiner finds that Weiskopf "clearly shows an improvement over the teachings of Yeung et al., as the teachings of Weiskopf et al., provides a clear advantage of derivatizing vitamin D

analogues with PTAD." *Id.* This is well-supported by the record. Weiskopf teaches as follows:

> Atmospheric pressure ionization methods, such as electrospray and atmospheric pressure chemical ionization (APCI), alleviate the thermal degradation problems encountered with GC/MS. Used with liquid chromatography (LC), these ionization techniques have permitted the low-level quantitation of vitamin D analogue metabolites from biological fluids and their structural identification by MS/MS. Underivatized vitamin D analytes, as with many steroidal compounds, provide excellent sensitivity by APCI. **Sensitive electrospray ionization (ESI) of vitamin D analytes generally requires the addition of a tagging agent to provide suitable sites for protonation or cationization**. Because a cisoid diene system is a feature common to most vitamin D compounds, dienophilic **reagents such as triazolinediones are particularly attractive**. Triazolinediones react with the C-10--19: C-5--6 diene by Diels--Alder cycloaddition (Fig. 2), and thereby derivatize vitamin D analytes in a structurally selective fashion, allowing their facile identification and isolation from other interferents in the analysis. While a number of triazolinedione variants for mass spectrometry have been described in the analytical literature, **our laboratory has adopted 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) as the reagent of choice**.

Weiskopf 72 (internal citations omitted). Thus, Weiskopf teaches to use PTAD-derivatized vitamin D analytes for electrospray ESI. Accordingly, Appellant has not shown that, at the time of application, there was widespread skepticism toward use of ESI and/or APCI to quantitate derivatized vitamin D metabolites. Nor has Appellant shown that any cited reference "teaches away" from the use of ESI or APCI to quantitate derivatized vitamin D metabolites.

Third, as best understood, Appellant argues that the claims are not obvious because mass spectrometry analysis of an analyte in a complex sample matrix is unpredictable and requires significant inventive effort. Appeal Br. 7–9. In support, Appellant cites to several journal articles said to "evidence the unpredictability of ionization efficiency in mass spectrometric methods across sample types." *Id.* at 8. Appellant concludes "[g]iven the well-recognized unpredictability of mass spectrometric methods with complex biological samples, therefore, such a disclosure does not render the claimed subject matter obvious over the prior art." *Id.* at 9.

The thrust of Appellant's argument appears to be that a person of ordinary skill in the art would not have been led to the present invention by the teachings of the cited art because of certain inherent unpredictability therein. By implication, Appellant argues that ordinary artisans are incapable of practicing the art. Appellant, however, has not explicitly addressed the level of ordinary skill in the art. *See Graham v. John Deere Co.*, 383 U.S. at 17. Accordingly, Appellant has not shown that one of ordinary skill would have been incapable of implementing the teachings of Clarke and Weiskopf.

Further, Clarke teaches that "[t]he skilled artisan will understand that the choice of ionization method can be determined based on the analyte to be measured, type of sample, the type of detector, the choice of positive versus negative mode, etc." Clarke ¶ 39. Similarly, Singh teaches that a "sample can be treated to remove components that could interfere with the mass spectrometry technique." Singh ¶ 20. Singh further teaches that "[a] variety of extraction and analytical columns with appropriate solvent mobile phases

and gradients can be chosen by those having ordinary skill in the art." *Id.* ¶ 23.

In addition, the Examiner finds that one of the references relied upon by Appellant, Fiehn, "teaches that chemical derivatization can be utilized as a means of **overcoming the stated drawbacks of ion suppression**." Answer 5 (emphasis added). This finding is not rebutted. Further, Weiskopf teaches that triazolinediones "derivatize vitamin D analytes in a structurally selective fashion, allowing their facile identification and **isolation from other interferents**." Weiskopf 72 (emphasis added). The above indicates that the stated ion suppression problem is not applicable to the Examiner's hypothetical combination.

In view of the foregoing, we determine that Appellant has not shown reversible error in the rejection of claim 1. As Appellant relies upon the same arguments in support of its appeal of the rejection of claims 2–12 and 14–23, we determine that Appellant has not shown reversible error in the rejection of those claims.

Rejection 2. The Examiner rejects claims 13 and 24–38 as obvious over Clarke in view of Weiskopf and further in view of Singh. Final Act. 5–7. Appellant relies upon the same arguments described above in support of its appeal of the rejection of claims 13 and 24–38. Appeal Br. 7–9. As we have found such arguments not to be persuasive, we determine that Appellant has not shown reversible error with regard to the rejection of claims 13 and 24–38.

CONCLUSION

The Examiner's rejections are affirmed.

In summary:

Claims Rejected	35 U.S.C. §	Reference(s)/Basis	Affirmed	Reversed
1–12, 14–23	103(a)	Clarke, Weiskopf	1–12, 14–23	
13, 24–38	103(a)	Clarke, Weiskopf, Singh	13, 24–38	
Overall Outcome			1–38	

No time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED