

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

LABORATORY CORPORATION OF AMERICA HOLDINGS,
Petitioner,

v.

QUEST DIAGNOSTICS INVESTMENTS LLC,
Patent Owner.

IPR2019-01425
Patent 8,101,427 B2

Before ULRIKE W. JENKS, SUSAN L. C. MITCHELL, and
ROBERT A. POLLOCK, *Administrative Patent Judges*.

POLLOCK, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining All Challenged Claims Unpatentable
35 U.S.C. § 318(a)

I. Introduction

This is a Final Written Decision in an *inter partes* review challenging the patentability of claims 1–11 of U.S. Patent No. 8,101,427 B2 (“the ’427 patent,” Ex. 1001). We have jurisdiction under 35 U.S.C. § 6.

Petitioner has the burden of proving unpatentability of a claim by a preponderance of the evidence. 35 U.S.C. § 316(e) (2018). Having reviewed the arguments of the parties and the supporting evidence, we find that Petitioner has demonstrated by a preponderance of the evidence that the challenged claims are unpatentable.

A. Procedural Background

Laboratory Corporation of America Holdings (“Petitioner”) filed a Petition for an *inter partes* review of claims 1–11 of the ’427 patent. Paper 1 (“Pet.”). Quest Diagnostics Investments LLC (“Patent Owner”) timely filed a Preliminary Response. Paper 7 (“Prelim. Resp.”). In view of the then-available, preliminary record, we concluded that Petitioner satisfied the burden, under 35 U.S.C. § 314(a), to show that there was a reasonable likelihood that Petitioner would prevail with respect to at least one of the challenged claims. Accordingly, on behalf of the Director (37 C.F.R. § 42.4(a) (2018)), and in accordance with *SAS Inst. Inc. v. Iancu*, 138 S. Ct. 1348, 1353 (2018) and the Office’s Guidance on the Impact of SAS on AIA Trial Proceedings (Apr. 26, 2018),¹ we instituted an *inter partes* review of claims 1–11 of the ’427 patent on all the asserted grounds. Paper 9 (“Inst. Dec.”), 25.

¹ <https://www.uspto.gov/patents-application-process/patent-trial-and-appeal-board/trials/guidance-impact-sas-aia-trial>.

B. Real Parties-in-Interest

Petitioner identifies itself, Laboratory Corporation of America Holdings, as the real party-in-interest. Pet. 1. According to Patent Owner, its real parties-in-interest are Quest Diagnostics Investments LLC and Quest Diagnostics Inc. Paper 5, 1.

C. Related Proceedings

According to the parties, the '427 patent is at issue in *Quest Diagnostics Investments LLC v. Laboratory Corp. of America Holdings*, No. 1:18-cv-01436-UNA (D. Del.). Paper 11; Paper 12, 2. Petitioner has also challenged U.S. Patent No. 8,409,862 in IPR2019-00738 (challenged claims found unpatentable); U.S. Patent No. 7,972,867 in IPR2019-01517 (institution denied on the merits); and U.S. Patent No. 7,972,868 in IPR2019-01618 (terminated after institution upon request of the parties). Paper 11; Paper 12, 2. The involved patents address subject matter similar to that of the '427 patent but are not related by chain of priority.

D. The '427 Patent and Relevant Background

1. Specification

The '427 patent is directed to detecting vitamin D metabolites using mass spectrometry. Ex. 1001, Abstract. According to the Specification, "Vitamin D is an essential nutrient with important physiological roles in the positive regulation of calcium (Ca^{2+}) homeostasis." *Id.* at 1:21–23. The two forms of vitamin D, vitamin D₂ (derived from fungal and plant sources) and vitamin D₃ (produced in animals upon exposure to sunlight), must undergo metabolic activation to form bioactive metabolites. *Id.* at 1:23–55. The initial step of vitamin D₃ activation humans,

occurs primarily in the liver and involves hydroxylation to form the intermediate metabolite 25-hydroxyvitamin D₃ (25-hydroxycholecalciferol; calcifediol; 25OHD₃). Calcifediol is the major form of vitamin D₃ in the circulation. Circulating 25OHD₃ is then converted by the kidney to 1,25-dihydroxyvitamin D₃ (calcitriol; 1,25(OH)₂D₃), which is generally believed to be the metabolite of vitamin D₃ with the highest biological activity.

Id. at 1:32–41. Once absorbed, Vitamin D₂ undergoes a similar pathway of metabolic activation involving the sequential generation of 25-hydroxyvitamin D₂ (25OHD₂) and 1,25-dihydroxyvitamin D₂ (1,25(OH)₂D₂). *Id.* at 1:47–50; *see also* Ex. 1002 ¶¶ 19–20 (reviewing Vitamin D structure and metabolism).

According to the Specification, “[m]easurement of vitamin D, the inactive vitamin D precursor, is rare in clinical settings and has little diagnostic value.” Ex. 1001, 1:56–57. But “serum levels of 25-hydroxyvitamin D₃ and 25-hydroxyvitamin D₂ (total 25-hydroxyvitamin D; ‘25OHD’) are a useful index of vitamin D nutritional status and the efficacy of certain vitamin D analogs” and “measurement of 25OHD is commonly used in the diagnosis and management of disorders of calcium metabolism.” *Id.* at 1:58–64.

The ’427 Specification, accordingly, discloses methods using mass spectrometry to detect the amount of vitamin D metabolites, such as 25OHD, in a sample. *Id.* at 2:39–41, 2:66–3:1. The Specification teaches that samples, including biological samples (e.g., blood, plasma, serum, etc.), may be purified prior to mass spectrometry analysis. *Id.* at 6:9–27. Purification includes protein precipitation and other protein purification methods known in the art, followed by chromatography, preferably liquid

chromatography (“LC”) including high performance liquid chromatography (“HPLC”), and chiral chromatography. *Id.* at 6:25–8:27.²

The disclosed method further includes steps of “[(1)] ionizing the purified vitamin D metabolite and [(2)] detecting the presence or amount of the ion, wherein the presence or amount of the ion is related to the presence or amount of the vitamin D metabolite in the sample.” *Id.* at 5:41–44. The method preferably employs tandem mass spectrometry, and the ionizing step includes ionizing a vitamin D metabolite to generate a protonated and dehydrated precursor ion, isolating the precursor ion, and fragmentation (i.e., effecting a collision between the isolated precursor ion and an inert collision gas to produce at least one fragment ion). *Id.* at 5:45–59. In preferred embodiments, “at least one fragment ion is detected, wherein the presence or amount of the precursor and/or at least one fragmentation ion is related to the presence or amount of the vitamin D metabolite in the sample.” *Id.* at 5:60–63.

2. Challenged Claims

The ’427 patent includes 23 claims, of which claims 1–11 are challenged in the Petition. Pet. 8. Claims 2–11 depend, directly or indirectly, from claim 1, which recites:

1. A method for determining the amount of a vitamin D metabolite in a sample by tandem mass spectrometry, comprising:
 - (a) generating a protonated and dehydrated precursor ion of said vitamin D metabolite;

² In this respect, the Specification states that the method preferably does not employ gas chromatography and that protein precipitation obviates the need for high turbulence liquid chromatography (“HTLC”). Ex. 1001, 6:49–64.

- (b) generating one or more fragment ions of said precursor ion; and
- (c) detecting the amount of one or more of said ions generated in step (a) or (b) or both and relating the detected ions to the amount of said vitamin D metabolite in said sample.

Ex. 1001, 20:44–54. Claims 2–9 are directed to pre-ionization purification steps. Claims 10 and 11 specify that the vitamin D metabolite is 25-hydroxyvitamin D₃ or 25-hydroxyvitamin D₂, respectively.

E. Asserted Grounds of Unpatentability

Petitioner asserts five grounds of unpatentability (Pet. 8):

Ground	Claims Challenged	Statutory Basis	Reference(s)
1	1–5	§ 103(a) ³	Voyksner ⁴
2	6, 7	§ 103(a)	Voyksner and Kissmeyer ⁵
3	8	§ 103(a)	Voyksner and Kamao ⁶

³ The Leahy-Smith America Invents Act, Pub. L. No. 112-29, 125 Stat. 284 (2011) (“AIA”), amended 35 U.S.C. § 103. Because the challenged claims of the ’427 patent have an effective filing date before the effective date of the applicable AIA amendments, we refer to the pre-AIA versions of 35 U.S.C. § 103 throughout this Decision.

⁴ Robert D. Voyksner *et al.*, *Improvements in LC/Electrospray Ion Trap Mass Spectrometry Performance Using an Off-Axis Nebulizer*, 71 AMERICAN CHEMICAL SOCIETY 1441–1447 (1999). Ex. 1004.

⁵ Anne-Marie Kissmeyer *et al.*, *Sensitive analysis of 1 α ,25-dihydroxyvitamin D₃ in biological fluids by liquid chromatography-tandem mass spectrometry*, 935 J. OF CHROMATOGRAPHY A 93–103 (2001). Ex. 1005.

⁶ Maya Kamao *et al.*, *C-3 Epimerization of Vitamin D₃ Metabolites and Further Metabolism of C-3 Epimers*, Manuscript M311473200, JBC PAPERS IN PRESS 1 (2004). Ex. 1006.

Ground	Claims Challenged	Statutory Basis	Reference(s)
4	9	§ 103(a)	Voyksner, Watson, ⁷ and Vogeser ⁸
5	10, 11	§ 103(a)	Voyksner, Watson, and Rainbow ⁹

In support of its patentability challenges, Petitioner relies on, *inter alia*, the Declaration of Dr. Robert L. Fitzgerald. Ex. 1002.

II. ANALYSIS

A. Legal Standards

“In an [*inter partes* review], the petitioner has the burden from the onset to show with particularity why the patent it challenges is unpatentable.” *Harmonic Inc. v. Avid Tech., Inc.*, 815 F.3d 1356, 1363 (Fed. Cir. 2016) (citing 35 U.S.C. § 312(a)(3)) (“[R]equiring [*inter partes* review] petitions to identify ‘with particularity . . . the evidence that supports the grounds for the challenge to each claim.’”). This burden of persuasion never shifts to Patent Owner. *See Dynamic Drinkware, LLC v. National Graphics, Inc.*, 800 F.3d 1375, 1378 (Fed. Cir. 2015) (discussing the burden of proof in *inter partes* review).

⁷ Watson *et al.*, *Analysis of Vitamin D and Its Metabolites using Thermospray Liquid Chromatography/Mass Spectrometry*, 5 BIOMEDICAL CHROMATOGRAPHY 153–160 (1991). Ex. 1007.

⁸ Michael Vogeser, *Liquid Chromatography-Tandem Mass Spectrometry – Application in the Clinical Laboratory*, 41 CLINICAL CHEMISTRY AND LABORATORY MEDICINE 115–252 (2003). Ex. 1009.

⁹ S. Rainbow *et al.*, *The Analysis of Vitamin D Analogues by Atmospheric Pressure Ionization Coupled to Triple Quadrupole Mass Spectrometry*, Applied Biosystems, (Poster No. M-045, PROCEEDINGS OF THE 51ST ASMS CONFERENCE ON MASS SPECTROMETRY AND ALLIED TOPICS) (2003). Ex. 1008.

A claim is unpatentable under 35 U.S.C. § 103(a) if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which that subject matter pertains. *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). The question of obviousness is resolved on the basis of underlying factual determinations including: (1) the scope and content of the prior art; (2) any differences between the claimed subject matter and the prior art; (3) the level of ordinary skill in the art; and (4) objective evidence of nonobviousness. *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1, 17–18 (1966).

In analyzing the obviousness of a combination of prior art elements, it can be important to identify a reason that would have prompted one of skill in the art “to combine . . . known elements in the fashion claimed by the patent at issue.” *KSR*, 550 U.S. at 418. A precise teaching directed to the specific subject matter of a challenged claim is not necessary to establish obviousness. *Id.* Rather, “any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.” *Id.* at 420. Accordingly, a party that petitions the Board for a determination of unpatentability based on obviousness must show that “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1381 (Fed. Cir. 2016) (quotations and citations omitted).

B. Level of Ordinary Skill in the Art

In determining the level of skill in the art, we consider the type of problems encountered in the art, the prior art solutions to those problems, the rapidity with which innovations are made, the sophistication of the technology, and the educational level of active workers in the field. *See Custom Accessories, Inc. v. Jeffrey-Allan Industries, Inc.*, 807 F.2d 955, 962 (Fed. Cir. 1986); *see also Orthopedic Equip. Co. v. United States*, 702 F.2d 1005, 1011 (Fed. Cir. 1983).

Petitioner contends that a person of ordinary skill in the art as of the relevant date “would have an advanced degree (e.g., M.S. or Ph.D.) in chemistry or a related discipline, such as pharmacology or biochemistry, and at least 2–3 years of experience in a clinical or research laboratory” as well as knowledge and experience with relevant extraction methods, MS, HPLC, and “the use of such methods for determining levels of analytes in biological samples.” Pet. 20; *see* Reply 1–2; Ex. 1002 ¶ 12. Petitioner also states a “person of ordinary skill in the art also would have knowledge regarding what parameters would be suitable to adapt for the optimization of extraction, HPLC, and/or MS to meet the requirements of a particular laboratory test.” Pet. 20.

Patent Owner proposes a similar definition, stating that one of ordinary skill in the art would have had “a bachelor’s degree in chemistry, medicinal chemistry, biochemistry, pharmaceuticals, or a related discipline and at least three to four years of experience using chromatography to perform analytical assays to characterize organic and/or biological materials,” or, in the alternative, “a lesser amount of experience, such as one to two years of experience following a master’s degree or a few months to a year of experience following a doctorate.” PO Resp. at 14–15 (citing

Ex. 2009 ¶ 31). Also similar to the Petitioner’s definition, Patent Owner contends that the skilled artisan “would be familiar with the application of mass spectrometry, including tandem mass spectrometry, in conjunction with High Performance Liquid Chromatography (HPLC) to perform those analytical assays.” *Id.* at 14. In this respect, we find Patent Owner’s somewhat broader definition reasonable and adopt this definition.

Patent Owner also contends, however, that the ordinarily skilled artisan would not necessarily have had “knowledge regarding what parameters would be suitable to adapt for the optimization of extraction, HPLC, and/or MS to meet the requirements of a particular laboratory test,” as set forth in Petitioner’s proposed definition. *Id.* at 50. Patent Owner contends that this would apply, in particular, to LC/MS-MS assays for detecting and quantitating vitamin D metabolites because this was a new technique used by “a low percentage of artisans.” *Id.* (citing Ex. 2009 ¶¶ 33–34).

We do not find this argument persuasive for the reasons set forth on page 1–3 of Petitioner’s Reply and in light of the prior art of record. *See* Reply 1–3 (citing, *e.g.*, Ex. 1002 ¶ 12 (Dr. Fitzgerald’s expert report); Ex. 1048 ¶ 128 (Dr. Sluss’ testimony in IPR2019-00738 that “[a] POSITA [person of ordinary skill in the art] would have considered optimizing the chosen [HPLC/MS-MS] techniques and methods to be a normal part of setting up and conducting diagnostic assays”)¹⁰; Ex. 1044 124:21–125:5 (Dr.

¹⁰ The ’427 patent claims a priority date of May 25, 2011. Dr. Sluss’s testimony in IPR2019-00738 relates to US. Pat. No. 8,409,862 B2, which issued from a chain of continuation applications first filed on December 2, 2003. Because Dr. Sluss’s testimony applies to the understanding of those of skill in art well before the critical date for the ’427 patent, we find it relevant here.

Sluss’s testimony from IPR2019-00738 that a bench technician with three or four years of experience in LC/MS/MS would have been able to optimize the method as part of setting up a diagnostic assay); Ex. 1004 (disclosing the use of three variants of LC/MS/MS for determining dihydroxyvitamin D3 in plasma); Ex. 1009 (reviewing use and method development of LC/MS-MS in clinical chemistry); Ex. 1008 (LC-MS/MS “can be used to routinely assay and quantify plasma levels of vitamin D2 & D3 metabolites”). In any event, Patent Owner contends that it would prevail under either parties’ definition. PO Resp. 15.

In view of the above, we adopt, in part, Patent Owner’s definition of one of ordinary skill in the art with the caveat that the skilled artisan would have knowledge regarding what parameters would be suitable to adapt for the optimization of LC/MS/MS in diagnostic assays.

C. Claim Construction

We interpret a claim “using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. [§] 282(b).” 37 C.F.R. § 42.100(b) (2019). Under this standard, we construe the claim “in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent.” *Id.* Furthermore, we need only construe the claims to the extent necessary to determine whether the challenged claims are unpatentable. *See Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co. Ltd. v. Matal*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) (“[W]e need only construe terms ‘that are in controversy, and only to the extent necessary to resolve the controversy.’” (quoting *Vivid Techs., Inc. v. Am. Sci. & Eng’g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))).

1. *“relating the detected ions to the amount of said vitamin D metabolite in said sample”*

In its Preliminary Response, Patent Owner argued that the claim phrase “relating the detected ions to the amount of said vitamin D metabolite in said sample,” recited in independent claim 1, “requires a quantitative correlation to be made between the ions detected and the level of vitamin D metabolite present in said sample.” Prelim Resp. 9. Patent Owner further stated that, in related district court litigation, Petitioner agreed that a quantitative correlation is required and offered the construction “quantifying the concentration of said vitamin D metabolite in said sample based on the detected ions” for this phrase. *Id.* at 9–10 (citing Ex. 2002, 23). Considering Patent Owner’s arguments, we provisionally construed “relating the detected ions to the amount of said vitamin D metabolite in said sample,” to mean “quantifying the concentration of a vitamin D metabolite in the sample based on the detected ions.” Inst. Dec. 10. Patent Owner makes similar arguments in the Patent Owner Response. PO Resp. 15–16.

Petitioner, in turn, contends that in the related district court litigation, Patent Owner argued that this language did not require construction. Reply 4 (citing Ex. 2025, 1–2, 11–12). According to Petitioner, [t]he court found the phrase “relating the detected ions to the amount of said vitamin D metabolite in said sample,” although a quantitative measure, does not require **“quantifying the concentration** of a vitamin D metabolite in the sample based on the detected ions.” *Id.* (citing Ex. 2025, 2 n.2, 11–12).

In setting forth its construction of “relating the detected ions to the amount of said vitamin D metabolite in said sample,” the district court expressly “reject[ed Petitioner’s] attempt to read in a requirement that the concentration of vitamin D metabolite be quantified,” (Ex. 2025, 2 n.2), but

subsequently stated that essentially the same concept in a claim specifying a particular metabolite “is quantitative, i.e., describing measuring the amount of 25-hydroxyvitamin D2” (*id.* at 11). As we understand the district court’s position, the language at issue is “quantitative” insofar as it requires comparing the amount of detected ions to the amount of vitamin D metabolite in the sample to some reference, but does not require a further step of calculating/quantifying a precise concentration of the metabolite.

Although we find the district court’s construction consistent with the claim’s use of the general term “relating” rather than the more precise term, “quantifying,” we also look to the overall structure of the claim 1, and in particular, the preamble language “[a] method for determining the amount of vitamin D metabolite in a sample.” In this respect, Patent Owner implies that we should accord weight to the preamble language, and equate “determining” with “quantifying.” PO Resp. 15–16. In particular, Patent Owner argues that “all terms” of the challenged claims should be afforded their plain and ordinary meaning and cites the preamble of claim 1 in support of its position that the disputed “relating” language “involves **quantifying** a vitamin D metabolite.” *Id.* (citing, e.g., Ex. 1001, 20:43–46). In contrast, Petitioner takes no position as to whether the preamble is limiting. *See* Pet. 24 (claim chart evidence for preamble), 25 (arguing, “[t]o the extent the preamble is limiting, Voyksner discloses or suggests every feature”).

We agree with Patent Owner that the preamble of claim 1 is limiting. “If the claim preamble, when read in the context of the entire claim, recites limitations of the claim, or, if the claim preamble is ‘necessary to give life, meaning, and vitality’ to the claim, then the claim preamble should be

construed as if in the balance of the claim.” *Pitney Bowes, Inc. v. Hewlett Packard Co.*, 182 F.3d 1298, 1305 (Fed. Cir. 1999) (citations omitted). In this case, the vitamin D metabolite recited in the preamble provides antecedent basis for “said vitamin D metabolite” in steps (a) and (c) of the claim body.

The “method for determining” language also recited in the preamble suggests a quantitative step that provides context for the “relating” language of step (c). Moreover, the Specification refers to a method for “detecting the presence or amount of one or more vitamin D metabolites in a sample” that involves “relating the detected ions to the presence or amount of the vitamin D metabolite in the sample.” Ex. 1001, 2:45–51, 3:1–13. In context, and noting that the claim body recites the amount, rather than the presence of vitamin D metabolite, we agree with Patent Owner that “relating the detected ions to the amount of said vitamin D metabolite in said sample,” means “quantifying the concentration of a vitamin D metabolite in the sample based on the detected ions.” *See* PO Resp. 15–16; Inst. Dec. 10. Consistent with our understanding of the District Court’s construction, we nevertheless, emphasize that neither the plain words of the claim, nor our construction, requires any particular level of precision and, in fact, encompasses the determination of even relative amounts of a vitamin D metabolite, i.e., determining whether one sample contains quantitatively more vitamin D metabolite than another.

2. “a vitamin D metabolite”

The parties appear to disagree as to whether “a vitamin D metabolite” in the preamble—and its descendant, “said vitamin D metabolite” in the body of claim 1—are limited to a single vitamin D metabolite or encompass one or more vitamin D metabolites. *See, e.g.*, Reply 6–7 (Petitioner arguing

that the claims encompass detection of more than a single vitamin D metabolite); Tr. 33:16–34:4 (Patent Owner stating that a metabolite is a single, unique species and ordinary meaning applies).

Pertinent to this issue, the Federal Circuit has established that, “as a general rule, the words ‘a’ or ‘an’ in a patent claim carry the meaning of ‘one or more.’” *TiVo, Inc. v. EchoStar Comms. Corp.*, 516 F.3d 1290, 1303 (Fed. Cir. 2008) (quoting *Baldwin Graphic Sys., Inc. v. Siebert, Inc.*, 512 F.3d 1338, 1342 (Fed. Cir. 2008)). This “is particularly true when those words are used in combination with the open-ended antecedent ‘comprising.’” *Baldwin Graphic Sys., Inc.*, 512 F.3d at 1342. “The exceptions to this rule are extremely limited: a patentee must evince a clear intent to limit ‘a’ or ‘an’ to ‘one.’” *Id.* (internal quotation marks and citation omitted). “An exception to the general rule arises *only* ‘where the language of the claims themselves, the specification, or the prosecution history necessitate a departure from the rule.’” *Communique Lab., Inc. v. LogMeIn, Inc.*, 687 F.3d 1292, 1297 (Fed. Cir. 2012) (finding ambiguity in the specification insufficient to overcome the general rule).

As an initial matter, we note that the first use of “a vitamin D metabolite” in claim 1 occurs in the preamble and, thus, prior to the “comprising” language. *See Baldwin Graphic Sys., Inc.*, 512 F.3d at 1342 (general rule particularly applicable where the singular “a” or “an” is “used in combination with the open-ended antecedent ‘comprising’”). Claim 1, nonetheless, twice refers to “said vitamin D metabolite” in the claim body, and after the comprising term. As noted in *Baldwin*, “subsequent use of definite articles ‘the’ or ‘said’ in a claim to refer back to the same claim term does not change the general plural rule, but simply reinvokes that non-

singular meaning.” *Baldwin Graphic Sys.*, 512 F.3d at 1342. Accordingly, the structure of claim 1 does not weaken the presumption that “a vitamin D metabolite” is not limited to a single vitamin D metabolite.

Neither party points us to the prosecution history as illuminating the meaning of this term. With respect to the Specification, both parties point to the paragraph at column 3, lines 32–36. Patent Owner focuses on the language “[a]s used herein, the term ‘vitamin D metabolite’ refers to any chemical species that may be found in the circulation of an animal which is formed by a biosynthetic or metabolic pathway for vitamin D or a synthetic vitamin D analog.” Sur-reply 8 (quoting Ex. 1001, 3:32–35) (emphasis omitted). Patent Owner appears to argue that the identity of a particular vitamin D metabolite species must be known “because the thrust of the patent is to use particular ions derived from those particular metabolite species to help with identification and quantification.” *Id.* at 8–9 (citing Ex. 1001, 10:1–9). We do not read the passages cited by Patent Owner as evincing a clear intent to limit “a” vitamin D metabolite to a single species. *See Baldwin Graphic Sys., Inc.*, 512 F.3d at 1342.

Petitioner, by contrast, focuses on subsequent language in the same paragraph which states: “In particularly preferred embodiments, the vitamin D metabolite is ***one or more compounds*** selected from the group consisting of 25-dihydroxyvitamin D₃, 25-hydroxyvitamin D₂, 1,25-dihydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₂.” Reply 6 (quoting Ex. 1001, 3:32–45). The language cited by Petitioner is most plausibly read as indicating that the claim encompasses mixtures comprising 25(OH)₂D₃, 25(OH)₂D₂, 1,25(OH)₂D₃, and 1,25(OH)₂D₂. But even if the drafters used the plural “one or more compounds” only in reference to a plurality of non-claimed

embodiments, the language from the Specification, taken as a whole, does not overcome the presumption that “a vitamin D metabolite” refers to one or more vitamin D metabolites.

We also do not read the claims of the ’427 patent as unambiguously limiting “a vitamin D metabolite” to a single species. Claim 1 recites “[a] method for determining the amount of a vitamin D metabolite in a sample by tandem mass spectrometry, comprising: . . . relating the detected ions to the amount of said vitamin D metabolite. . . .” Neither party argues that the claimed method could not be performed on a sample containing more than one vitamin D metabolite. To the contrary Patent Owner admits that “[t]he patent does not preclude using this method to quantitate multiple different vitamin D metabolites simultaneously.” Sur-reply 9; *see also* claim 13 (directed to “determining the amounts of two or more vitamin D metabolites”).

For the reasons set forth above, we interpret “a vitamin D metabolite” as encompassing one or more vitamin D metabolites.

D. Overview of Asserted References

1. Voyksner (Ex. 1004)

According to Voyksner, “[d]etection of lipophilic vitamins such as vitamin D₃ and their analogues [e.g., dihydroxyvitamin D₃] proves to be a challenge due to the low levels that result in physiological effects and the need to have accurate and specific analytical methods for their detection.” Ex. 1004, 1442. To compare LC/electrospray ion trap mass spectrometry performance using an off-axis nebulizer with prior techniques, Voyksner assays dihydroxyvitamin D₃ in plasma by liquid chromatography followed by each of three variants of tandem mass spectrometry (“LC/MS/MS”): ion

trap with on-axis nebulizer, ion trap with off-axis nebulizer, and triple quadrupole. *Id.* at 1441–1442,¹¹ 1446 (Table 1).¹² All three methods generated a protonated and dehydrated precursor ion of the dihydroxyvitamin D₃, [M + H - H₂O]⁺. *Id.* at 1446–1447 (“parent ion at *m/z* 399”).¹³ Voyksner explains that subsequent collision-induced decomposition (CID) of the parent ion produced fragments having mass-to-charge ratio *m/z* 381 for the off-axis nebulization method and *m/z* 135 and 213 for the triple-quadrupole method. *Id.*

In Figure 8a, reproduced below, Voyksner discloses ion chromatograms for the determination for 3 ng/mL level of dihydroxyvitamin D₃ in plasma using the two LC/MS/MS ion trap configurations.

¹¹ We refer to Voyksner’s native pagination throughout.

¹² Voyksner notes that its “initial comparison of capabilities between off-axis and on-axis nebulizer” methods was performed using lincomycin, adenosine, and betaine standards dissolved in an acetonitrile/water/acetic acid solvent. *Id.* at 1443.

¹³ Patent Owner does not dispute Petitioner’s contention that one of ordinary skill in the art would understand Voyksner’s *m/z* value of 399 as reflecting the molecular mass of dihydroxyvitamin D₃ (416.6 g/mol), minus a water molecule (18 g/mol), and plus a proton (1.007 g/mol). *See* Pet. 13, n. 5.

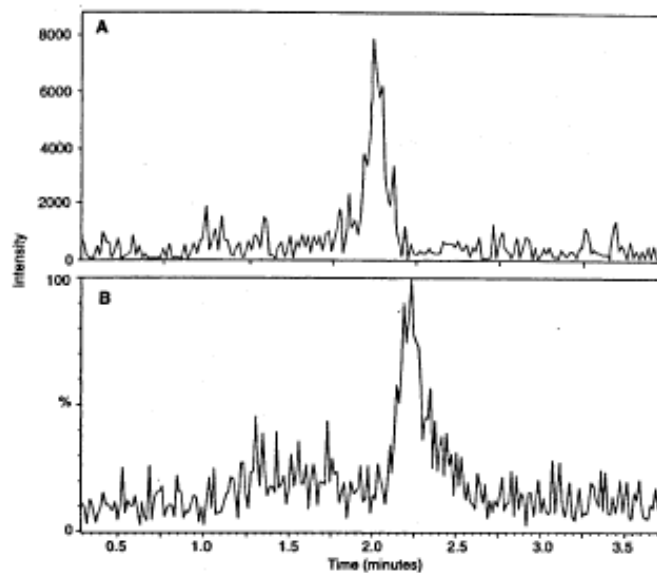


Figure 8 shows “LC/MS/MS ion chromatograms for the determination for 3 ng/mL level of dihydroxyvitamin D₃ in plasma by the off-axis nebulizer on the ion trap and the triple quadrupole.” *Id.* More specifically, panel 8A depicts results for the m/z 399–381 CID transition for the off-axis nebulizer ion trap, and panel 8B shows the m/z 399–135 CID transition for the triple quadrupole technique. *Id.* at 1447. According to Voyksner, “[t]he sensitivity of the two techniques were similar.” *Id.*

To address the relative precision of the three methods, Voyksner compares the relative standard deviation for measurements at 3 and 30 ng/ml in Table 1. *Id.* at 1446–1447. Voyksner reports the relative standard deviation for the measurement of dihydroxyvitamin D₃ was better at 30 ng/mL by the triple quadrupole compared to the off-axis nebulizer ion trap, whereas the two methods showed similar precision at 3 ng/mL. *Id.*

With respect to the two ion trap configurations, Voyksner teaches that the use of an off-axis nebulizer reduces total ion currents that enter the ion trap (through the reduction of charged residues) and results in an increase of

analyte signal as compared with results using on-axis nebulization. *Id.* at 1441–1442. Incorporation of the off-axis nebulizer thus improved the detection limit and precision for determination of the vitamin D metabolite dihydroxyvitamin D₃ in plasma as compared to on-axis nebulization. *See id.*

Voyksner concludes that the off-axis nebulizer ion trap permits dihydroxyvitamin D₃ to be “measured from a plasma extract down to 1 ng/mL with relative standard deviation of less than 8%.” *Id.* at 1447.

2. Kissmeyer (Ex. 1005)

Kissmeyer discloses methods for detecting and quantitating 1 α , 25-dihydroxyvitamin D₃ (“1 α , 25(OH)₂D₃”) in biological fluids using LC/MS/MS and electrospray ionization. Ex. 1005, Abstract. Kissmeyer discloses using protein precipitation prior to the LC/MS/MS analysis. Ex. 1005 §§ 2.3, 2.4.

3. Kamao (Ex. 1006)

Kamao discloses using chiral chromatography with ionization MS to detect epimers of vitamin D metabolites in rat plasma and cultured cells. Ex. 1006, Introduction. In particular, Kamao uses LC columns to separate 3-epi-25(OH)D₃ from 25(OH)D₃ based on differences in retention times, and detects the separated vitamin D metabolites with mass spectroscopy. *See, e.g., id.* at 8–9, 11–12, 19.

4. Watson (Ex. 1007)

Watson discloses analyzing vitamin D and its metabolites, e.g., 25-hydroxyvitamin D₃ (25(OH)D₃) and 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), using thermospray LC/MS (“TSP-LC/MS”) as compared to GC/MS (gas chromatography/mass spectrometry). Ex. 1007, Abstract, Fig. 1. Watson uses the detection of 1,25(OH)₂D₃ to “fine tune[]” the TSP-

LC/MS method “because [it] is the most biologically potent [vitamin D] metabolite and its concentration *in vivo* is normally three orders of magnitude lower than that of vitamin D₃ and 25(OH)D₃. Hence the detection limit for 1,25(OH)₂D₃ is an important test of any proposed comprehensive analytical approach for this group of compounds.” *Id.* at 7. Watson reports that “[u]nder conditions optimized to yield the maximum relative ion current at *m/z* 399 it was possible to detect 0.6 pmol of 1,25(OH)₂D₃.” *Id.* (citing Fig. 2). Watson concludes that TSP-LC/MS “should afford several advantages for analysis of vitamin D and its metabolites compared with gas chromatography/mass spectrometry (GC/MS)” (*id.* at 4), and “can be applied to *in vitro* and *in vivo* studies of metabolism of these compounds” (*id.* at 10).

5. Vogeser (Ex. 1009)

Vogeser is a review article discussing liquid chromatography-tandem mass spectrometry (LC-TMS, i.e., LC/MS/MS), “as an emerging technology in clinical chemistry.” Ex. 1009, Abstract. Vogeser discloses:

[t]he essential step in the development of LC-MS as a routine technique for the clinical laboratory was the introduction of collision-induced dissociation (CID) with a second quadrupole for analysis of the fragments generated in this way (“tandem”-MS): In these instruments, a first quadrupole selects the molecular (“parent”) ion of the respective analyte, which is then directed into a collision cell; here a very low flow of argon enters as the collision gas. By collision with these argon molecules the parent ions are disintegrated into several typical so-called “daughter” or “product ions[.]” These daughter ions can then be scanned according to their respective *m/z* ratio by a second quadrupole.

Id. at 119.

6. Rainbow (Ex. 1008)

Rainbow is a poster presented at the 51st ASMS Conference on Mass Spectrometry and Allied Topics in Montreal, Canada in June 2003. Reply 23–25 (citing Ex. 1002 ¶ 50; Ex. 1020; Ex. 1021 ¶¶ 3–6; Ex. 1043, Ex. 1046 ¶¶ 2–3).¹⁴

Rainbow discloses quantifying the plasma levels of 25-hydroxy vitamin D₂, 25-hydroxy vitamin D₃, and 1,25-hydroxy vitamin D₂ in a patient plasma sample by LC/MS/MS using atmospheric pressure ionization. Ex. 1008 (Abstract, Results). According to Rainbow, the poster shows “the method can be used to generate a metabolite profile for real patient plasma samples.” *Id.* (Introduction).

In the Materials & Methods section, Rainbow discloses its method of purifying plasma samples for HPLC and the HPLC equipment and parameters. After HPLC purification, “Autosampler MS/MS was carried out on an Applied Biosystems/MDS Sciex API 3000™ Triple Quadrupole Mass Spectrometer fitted with a TurboIonSpray® ion source. Data capture and analysis was carried out using Applied Biosystems/MDS Sciex Analyst® software.” *Id.* (Materials & Methods).

Rainbow further describes the construction of calibration curves using calibration samples comprising various concentrations of 25OHD₃ in

¹⁴ In our Institution Decision, we addressed Patent Owner’s argument that Ground 5 failed because Rainbow did not qualify as prior art to the ’475 patent. *See, e.g.*, Inst. Dec. 22–24. Subsequent to institution, we granted Petitioner’s motion to submit supplemental information relating to the public availability of the Rainbow reference. Paper 22 (granting Petitioner’s unopposed motion to submit Exhibits 1042 and 1043). Although earlier at issue, Patent Owner no longer contests that Rainbow is prior art. Tr. 5:20–26, 46:18–21; *see also* Reply 23 (Petitioner’s reasoned argument that Rainbow qualifies as prior art).

artificial plasma, which were subsequently spiked with known concentrations of commercially available 25OHD₂. *Id.* (“In order to produce a calibration curve for both 25-(OH) Vitamin D2 & D3, [the 25OHD₃-containing] solutions were spiked with a known concentration of 25-(OH) Vitamin D2 (Fluka).”). Rainbow presents representative chromatograms from the calibration runs along with 25OHD₂ and 25OHD₃ standard curves and a table of their underlying data.

Rainbow further discloses a chromatogram of extracted patient plasma sample showing a “full metabolic profile,” and including peaks labeled as corresponding to 25OHD₂, 1,25OHD₂, and 25OHD₃. *Id.* (Results). Rainbow concludes that the disclosed LC/MS/MS method “can be used to routinely assay and quantify plasma levels of vitamin D2 & D3 metabolites” and “enables clinical researchers to diagnose specific problems, to track the course of a disease and to monitor treatment and recovery in real time.” *Id.* (Conclusion).

E. Obviousness Over Voyksner alone (Ground 1) or in Combination with Kissmeyer (Ground 2), Kamao (Ground 3), and Watson and Vogeser (Ground 4)

As Ground 1, Petitioner challenges claims 1–5 as obvious over Voyksner. Pet. 20–34; Reply 5–19. Petitioner’s challenge includes a detailed mapping of the teachings of the reference to each limitation of the claims. Pet. 20–34. Patent Owner opposes. PO Resp. 18–40; Sur-reply 2–15.

For Ground 2, Petitioner similarly challenges claims 6 and 7 as obvious over Voyksner and Kissmeyer (Pet. 34–39); for Ground 3, Petitioner challenges claim 8 as obvious over Voyksner and Kamao (*id.* at 40–42); and for Ground 4, Petitioner challenges claim 9 as obvious over Voyksner, Watson, and Vogeser (*id.* at 43–47). In responding to these

Grounds, Patent Owner relies solely on its arguments with respect to Voyksner as applied to Ground 1, claim 1. PO Resp. 40. Because Patent Owner does not address the additional limitations of claims 2–9, we focus our analysis on independent claim 1, from which those claims depend.

Relying on the testimony of Dr. Fitzgerald, Petitioner argues that Voyksner discloses or renders obvious each limitation of claim 1. *See, e.g., id.* at 22–23, 29 (citing Ex. 1004, 5–11; Ex. 1002 ¶¶ 55–67). In particular, Petitioner argues that “Voyksner, in view of the general knowledge in the art, discloses detecting the amount of one or more of the precursor ion or fragment ions of a vitamin D metabolite and relating the detected ions to the amount of vitamin D metabolite in the sample, as in step (c) of claim 1.” *Id.* at 29 (citing Ex. 1004, 10–11; Ex. 1002, ¶¶ 63–64).

Patent Owner contends that Grounds 1–4 fail for two principal reasons. Patent Owner first argues that Voyksner’s reference to “dihydroxyvitamin D₃” is ambiguous and does not satisfy the “a vitamin D metabolite” limitation of claim 1. PO Resp. 19–29; Sur-reply 2–10. Second, Patent Owner argues, because Voyksner does not disclose “a vitamin D metabolite” it does not “relate the detected ions to the amount of said vitamin D metabolite in a sample,” as also required by independent claim 1. PO Resp. 29–39; Sur-reply 10–15; Tr. 36:8–38:5. For the reasons set forth below, Patent Owner’s arguments do not undermine Petitioner’s persuasive showing that the claims are obvious.

1. Whether Voyksner discloses or renders obvious “a vitamin D metabolite”

Patent Owner presents extensive evidence that “when they wanted to do so” those of ordinary skill in the art knew how to select and describe with specificity as many as five dihydroxyvitamin D₃ metabolites (1,25-

dihydroxyvitamin D₃, 24,25-dihydroxyvitamin D₃, 1,24-dihydroxyvitamin D₃, 25,26-dihydroxyvitamin D₃, and 23,25-dihydroxyvitamin D₃). PO Response 20–23 (citations omitted); *see also* Ex. 2004, 43:16–21 (Dr. Fitzgerald conceding that as of the critical date at least three different dihydroxyvitamin D₃ species were known). Patent Owner points out that Voyksner, however, refers generically to “dihydroxyvitamin D₃” without identifying a specific compound or even mentioning the word “metabolite.” Patent Owner argues that such usage “is unsurprising because Voyksner’s goal of testing nebulizer orientations had nothing to do with vitamin D metabolite quantification.” PO Resp. 19; Tr. 34:13–35:8. Given the thrust of Voyksner’s studies, we also find it unsurprising that Voyksner would employ a generic, shorthand designation for his test analyte.

Relying on the testimony of Dr. Sluss, however, Patent Owner contends that “‘dihydroxyvitamin D₃’ refers not to a *specific* vitamin D metabolite, but rather a *class* of vitamin D metabolites, which at the time of the ’427 invention would have included” the five species noted above that would have been known to one of skill in the art. *Id.* at 20 (citing Ex. 2009 ¶ 82). In failing to specify any particular species, Patent Owner contends that Voyksner does not disclose “a vitamin D metabolite,” as required by claim 1. *See id.* at 20. In support, Patent Owner points to the testimony of Petitioner’s expert, Dr. Fitzgerald, who reasoned that Voyksner was “most likely” using 1,25-dihydroxy D₃, but admitted that Voyksner could have been using one of several known metabolites or even, albeit “unlikely,” a mixture of species. *Id.* at 23–25 (citing Ex. 2004, 49:3–22, 50:13–18, 51:7–19); Ex. 2009 ¶ 89.

We do not find Patent Owner's argument persuasive. Considering Dr. Sluss's testimony that dihydroxyvitamin D₃ refers to a class of at least five known vitamin D metabolites, we infer that one of ordinary skill in the art would have recognized that Voyksner used one or more of these compounds as a standard for LC/MS/MS. Because it is undisputed that each compound making up the class of dihydroxyvitamin D₃ is a vitamin D metabolite, Voyksner's measurement of any one of the compounds listed by Dr. Sluss satisfies the claim limitation. *See* Reply 8, n.3. Moreover, to the extent Voyksner may have employed a mixture of vitamin D metabolites as Patent Owner suggests, claim 1's requirement for "a vitamin D metabolite" is similarly satisfied under our construction of this term. *See* section II.C.2, above.

We need not, however, rely on our construction of "a vitamin D metabolite" as encompassing more than one species. Although we agree with Dr. Sluss that dihydroxyvitamin D₃ generally refers to a small class of compounds, substantial evidence supports Petitioner's contention that one of ordinary skill in the art would have read Voyksner as using the term to refer to a single species. *See* Reply 7–12. As an initial matter, this finding is supported by Patent Owner's observation that Voyksner was not interested in quantitating vitamin D metabolites, per se, but merely employed a vitamin D metabolite as a challenging standard to assess improvements in LC/MS/MS technology. PO Resp. 19–20; Ex. 1002 ¶ 39; Ex. 2009 ¶¶ 61, 63 (citing Ex. 1004, 5–7). Given this context, we find it more likely that Voyksner used a single species of vitamin D metabolite rather than a mixture or class of compounds.

For the reasons advanced at pages 7–10 of the Petition, which we adopt, we agree with Petitioner that “it would have been nonsensical for Voyksner to use a mixture of different dihydroxyvitamin D₃ metabolites as this would have confounded the results.” The testimony of both parties’ experts supports this conclusion. As reproduced on page 24 of the Reply, Dr. Fitzgerald testified that it would have been “unlikely” for Voyksner to have used a mixture of vitamin D metabolites. Reply 24 (citing Ex. 2004, 51:17–19). Similarly, Dr. Sluss confirmed that in determining whether a method was suitable for quantitative measurement, it would be counterproductive to use a mixture of test compounds “because I can’t tell what the signal is coming from.” Ex. 1049, 114:6–15. We also note that, in the unlikely event Voyksner used a mixture of vitamin D metabolites, the individual species would have been separated by HPLC prior to ionization fragmentation. *See* Ex. 1002, 67 (citing Ex. 1004, 1443); Tr. 47:23–48:8; 1007, Table 1 (HPLC data showing different retention times for 25-hydroxyvitamin D₃, 1,25-dihydroxyvitamin D₃, and 24,25-dihydroxyvitamin D₃). Accordingly, even if Voyksner used a mixture of vitamin D metabolites that produced the same *m/z* fragments, the detected ionization signals would have been separated in time.

As noted above, we do not agree that Voyksner’s alleged failure to identify by name “a *specific* vitamin D metabolite” is dispositive. *See* PO Resp. 20. The evidence of record, however, indicates that Voyksner more likely than not used 1,25-dihydroxyvitamin D₃ as the reference sample. As noted in the Reply, Voyksner’s:

Figure 8 shows a single peak for the monitored protonated, dehydrated precursor to fragment transitions (399→381 and 399→135) using both ion trap and triple quadrupole MS/MS,

suggesting that a single dihydroxyvitamin D3 metabolite is being detected. EX1004, 11 (Figure 8).

Reply 9; *see also* Ex. 1004 Fig. 6 (further illustrating 399 to 381 m/z transition). As shown in Patent Owner's annotated Figure 1 from Watson, of the well-known vitamin D₃ metabolites, only 1,25(OH)₂D₃ and 24,25(OH)₂D₃ produce protonated, dehydrated ions having a mass to charge ratio of 399.¹⁵ Pet. 27–28; Ex. 2009 ¶¶ 82, 89; Tr. 36:8–37:10 (Patent Owner admitting that Voyksner optimized for detecting the m/z 399 ion). But as shown in Watson's Figure 1, the m/z 399 ion is the major peak for 1,25(OH)₂D₃, whereas the 24,25(OH)₂D₃ shows a major peak at m/z 417, with a much smaller signal at m/z 399. We find it unlikely that Voyksner would have focused its analysis of m/z 399→381 and m/z 399→135 transitions of using 24,25(OH)₂D₃ because that would require quantitating changes in an initially minor peak. We find it more likely that Voyksner's vitamin D₃ metabolite was, in fact 1,25(OH)₂D₃, because that compound shows a major protonated dehydrated ion peak at m/z 399 such that it would have been easier to quantitate the m/z 399→381 and m/z 399→135 transitions.

As outlined at pages 9 and 10 of the Reply, biological limitations of Voyksner's reagents lead us to the same conclusion. Petitioner notes that in comparing the precision of its dihydroxyvitamin D3 determinations,

Voyksner reports percent standard deviation data in Table 1 and observes that the dihydroxyvitamin D3 metabolite could be measured down to 1 ng/mL with a relative standard deviation of less than 8%. EX1004, 10 (Table 1), 11. To obtain these data, Voyksner spiked

¹⁵ Despite Dr. Sluss's testimony that "Watson implicates *at least two* different vitamin D metabolites for this m/z ratio," the record does not suggest that any other vitamin D metabolites produce such ions. *See* Ex. 2009 ¶ 89 (emphasis altered).

predetermined amounts of dihydroxyvitamin D₃ from 0.3 to 30 ng/mL into rat plasma. EX1004, 7

Reply 9. Petitioner reasons that because rat plasma levels of 24,25(OH)₂D₃ were known to be about 2–5 ng/ml, “of detection of 1 ng or 3 ng/mL of the spiked vitamin D₃ metabolite would have been obscured by endogenous levels of the more abundant 24,25(OH)₂D₃.” *Id.* at 9 (citing Ex. 2014, Tab. 3). Accordingly, “[i]t would have made no sense for Voyksner to measure 0.3 or 3 ng of 24,25(OH)₂D₃ against a background of 2-5 ng/mL of endogenous 24,25(OH)₂D₃.” *Id.*; *see also* Ex. 2009 ¶ 84 (Dr. Sluss discussion relative amounts of endogenous vitamin D metabolites in rat and human samples). In contrast, Petitioner notes that because the endogenous levels of 1,25(OH)₂D₃ in rat plasma were 0.005–0.1 ng/ml, and thus below the level of detection of Voyksner’s methods, this compound would be unlikely to interfere with the measurement of the spiked 1,25(OH)₂D₃. Reply at 9–10. Although Dr. Sluss recognizes that endogenous levels of dihydroxyvitamin D₃ metabolites in rat plasma can interfere with detection of some species, Patent Owner’s principle response appears to be that Voyksner “doesn’t tell us . . . where he sourced the rat plasma.” Ex. 2009 ¶¶ 88–89; Tr. 38:7–20. Absent any evidence that different strains of rats have vastly different levels of 24,25(OH)₂D₃ and 1,25(OH)₂D₃, we do not find Patent Owner’s argument persuasive.

For the above reasons, we conclude that one of ordinary skill in the art would have understood Voyksner to disclose “a vitamin D metabolite” and, in particular, 1,25-hydroxyvitamin D₃, along with protonated and dehydrated precursor ions thereof.

2. Whether Voyksner discloses or renders obvious “relating the detected ions to the amount of said vitamin D metabolite in a sample”

As set forth in section II.C.1, we construe “relating the detected ions to the amount of said vitamin D metabolite in said sample,” to mean “quantifying [at least relative to another sample] the concentration of a vitamin D metabolite in the sample based on the detected ions.” In addressing this limitation, Petitioner points to, for example, Table 1 and Figure 8 of Voyksner as describing detecting MS/MS fragment ions and relating the detected ions to the amount of vitamin D metabolite in the sample (i.e., either 3 or 30 ng/ml of dihydroxyvitamin D3). Pet. 28, 30. Petitioner also notes Voyksner’s conclusion that:

[u]sing the off-axis nebulizer, dihydroxyvitamin D3 could be measured from a plasma extract down to 1 ng/mL with relative standard deviation of less than 8%. The LC/MS/MS sensitivity and precision obtained for dihydroxyvitamin D3 in plasma using the off-axis nebulizer on the ion trap match closely the determinations made on a triple-quadrupole MS.

Id. at 30 (quoting Ex. 1004, 11).

Patent Owner contends that its “position is and always has been that Voyksner does perform a ‘quantitation.’” Sur-reply 11–12. In this respect, Patent Owner argues that, “[t]he problem with Voyksner is that it discloses recordings of ion signals from samples with **known** concentrations.” *Id.* at 12.

To the extent Patent Owner’s argues that Voyksner “only used samples where the analyte concentrations were established in advance,” we agree that Voyksner assayed “samples” comprising “rat plasma spiked with dihydroxyvitamin D3 from 0.3 to 30 ng/ml.” See PO Resp. 35; Exs. 1004, 1443, and 1446 (referencing “samples”). The claims before us, however, do

not require that the vitamin D metabolite, or the concentration thereof, in a sample is unknown, and thereby encompass reference samples. Moreover, the Specification expressly states that “[s]uitable samples include any sample that might contain the analyte of interest,” and expressly refers to “samples” as proteinaceous solutions spiked with known amounts of Vitamin D metabolites. *See* Ex. 1001, 6:9–11, 17:39–47 (“[S]tandard curve samples” made by adding known amounts of vitamin D metabolites to a Bovine Serum Albumin solution.), 17:61–67, and 18:49–54 (“[S]amples” comprising human serum spiked with known amounts of vitamin D metabolites.). Accordingly, the samples used in Voyksner are not excluded by the plain language of the claims and are clearly within the scope contemplated by the ’427 Specification.

Patent Owner further argues that Grounds 1–4 fail because one of ordinary skill in the art would not know the identity of the vitamin D metabolite(s) tested in Voyksner and, thus, use of this “nebulous . . . standard would have made the quantitation of individual levels of vitamin D metabolites impossible.” PO Resp. 4, 29–30; *see* Tr. 29:2–19. With respect to the precise identity of Voyksner’s analyte, we determined in section II.C.2, above, that “a vitamin D metabolite” encompasses one or more vitamin D metabolites, thus encompassing the use of a mixture of species. Moreover, as set forth in section II.E.1, above, the evidence of record indicates that, more likely than not, Voyksner’s “dihydroxyvitamin D₃” was a single vitamin D₃ metabolite and, more specifically, 1,25-hydroxyvitamin D₃. Nevertheless, to the extent Voyksner spiked its test samples with one or more *unspecified* vitamin D₃ metabolites, Voyksner, nevertheless, correlated the known amount of vitamin D₃ in the sample with the amount of ions

detected after mass spectrometry and quantified the concentration of a vitamin D metabolite in the sample based on the detected ions.

Patent Owner argues that Petitioner “conflate[s] the steps of ‘*detecting*’ the amount of precursor fragment ions with the different step of ‘*relating*’ the detected ions to the amount of vitamin D metabolite in the sample.” Sur-reply 12. We do not agree with Patent Owner.

With respect to the step of relating the ion signals to the amount of analyte present in a sample, we credit Dr. Fitzgerald’s description of Voyksner as “relating the amount of the precursor and/or fragment ions generated from the protonated, dehydrated precursor of dihydroxyvitamin D₃ to an amount of dihydroxyvitamin D₃ in the sample.” Ex. 1002

¶¶ 43, 64. In support of that opinion, Dr. Fitzgerald points to the data set forth in Voyksner’s Table 1, Figure 8, and the reference’s conclusions that using the off-axis nebulizer, “dihydroxyvitamin D₃ could be measured from a plasma extract down to 1 ng/mL with a relative standard deviation of less than 8%,” and that “the sensitivity and precision obtained for dihydroxyvitamin D₃ in plasma using the off axis nebulizer match closely the determinations made on a triple quadrupole MS.” *Id.* ¶ 43 (quoting Ex. 1004, 1447). According to Dr. Fitzgerald:

One of ordinary skill in the art would have understood that by determining and reporting the precision for the measurement of various concentrations of the vitamin D metabolite dihydroxyvitamin D₃, Voyksner was detecting the amount of one or more of either the precursor ion ($m/z = 399$) or the fragment ions (i.e., either $m/z = 381$ for ion trap MS/MS or $m/z = 135$ and $m/z = 213$ for triple quadrupole MS/MS) or both and

relating the detected ions to the amount of the vitamin D metabolite in the sample.

Id. ¶ 64. In sum, Voyksner’s determination that “dihydroxyvitamin D₃ could be measured . . . down to 1 ng/mL” makes clear that Voyksner could detect an amount of precursor fragment ions; relate the detected ions to the amount of vitamin D metabolite in a sample; and thereby obtain a quantitative value for the amount of vitamin D metabolite in the sample.

The testimony of the parties’ experts is also instructive on this point. According to Dr. Fitzgerald, Voyksner calculates percent relative standard deviations (a measure of precision) for the detection of samples having either 3 ng/mL or 30 ng/mL dihydroxyvitamin D₃ and “also notes that dihydroxyvitamin D₃ could be measured at concentrations as low as 1 ng/mL, with a relative standard deviation of less than 8%, using the off axis nebulizer.” Ex. 1002 ¶ 43 (citing Ex. 1004, Table 10–11). Although Dr. Sluss argues that Voyksner’s measurements are mere signal detection rather than quantitation, Dr. Sluss acknowledged at deposition that in determining the percent relative deviation, as done in Voyksner, “you would have to measure the ion generated by mass spec.” Ex. 2009 ¶¶ 99–103; Ex. 1049, 123:24–125:3. Accordingly, we find that in calculating the relative standard deviations for samples spiked with 3 ng/mL or 30 ng/mL analyte in Table 1, one of ordinary skill in the art would have understood Voyksner to have “relat[ed] the detected ions to the amount of said vitamin D metabolite in said sample,” as required by independent claim 1 and in light of our construction. *See* Pet 30; Reply 12–13 (citing Ex. 1004, 10–11; Ex. 1002 ¶ 64; Ex. 1049, 124:6–125:3); section II.C.1, above.

Patent Owner also contends that the Petition “does not address the accuracy of Voyksner’s methods” and one of skill in the art would have had

no way to know the amounts of dihydroxyvitamin D₃ metabolites Voyksner was measuring from its spiked samples because “any such signal would necessarily have been influenced by the endogenous levels known to exist in rat serum.” PO Resp. 31, 35–39 (further arguing that Voyksner did not account for the use of untreated rat plasma and failed to use validation controls).

As an initial matter, Patent Owner’s argument misses the mark insofar as none of the claims before us require a specific level of accuracy or precision. *See* Reply 14. Patent Owner provides no persuasive argument as to why we should import any such requirement into the claims.

With respect to Patent Owner’s technical criticism of Voyksner’s methods, it was known that the amount of 1,25(OH)₂D₃ in rat plasma was below the level of detection of Voyksner’s methods such that the endogenous metabolite would be unlikely to interfere with (let alone abrogate) the measurement of 1,25(OH)₂D₃ added to the sample. *See* section II.E.1, above. Moreover, methods for determining accuracy and precision of an assay, and the use of such assays to determine the amount of an unknown analyte in a sample were well known. Reply 15–18. Indeed, this basic understanding as applied to vitamin D metabolites was known in the art and expressly recognized in the ’427 Specification, which states:

Methods of generating and using such standard curves are well known in the art and one of ordinary skill is capable of selecting an appropriate internal standard. For example, an isotope of a vitamin D metabolite may be used as an internal standard, in preferred embodiments the vitamin D metabolite is a deuterated vitamin D metabolite, for example D-25OHD. Numerous other methods for relating the presence or amount of an ion to the

presence or amount of the original molecule will be well known to those of ordinary skill in the art.

Ex. 1001 9:38–48; *see also* Reply 16–17 (citing Ex. 1008 as evidence that one of ordinary skill in the art know how to prepare a standard calibration curve for the measurement of specific vitamin D metabolites); Ex. 1008 (disclosing “[a] method . . . to determine the plasma levels of 25-hydroxy vitamin D by LCMS/MS”); Ex. 1005 (disclosing methods for quantitating 25(OH)₂D₃ in biological fluids using LC/MS/MS); Ex. 1009, Abstract, 122 (discussing clinical use of LLC/MS/MS to quantify analytes in, e.g., “crude plasma or urine”); Tr. 18:1–23, 22:9–24. Accordingly, even if Voyksner did not expressly disclose quantifying an unknown amount of a vitamin D metabolite in a sample, one of ordinary skill in the art reading Voyksner would have considered it obvious to do so.

3. Conclusion as to grounds 1–4

We have reviewed the relevant record regarding Grounds 1–4. We find that Petitioner has demonstrated that the cited references teach or suggest each of the undisputed limitations, and provided a reasonable rationale for modifying and/or combining the cited art. We conclude that Petitioner has established by a preponderance of the evidence that claims 1–9 are obvious over the cited combinations of Voyksner, Kissmeyer, Kamao, Watson, and Vogeser in view of the knowledge of one of ordinary skill in the art.

F. Obviousness Over the Combined Teachings Voyksner, Watson, and Rainbow (Ground 5)

Claims 10 and 11 specify that the detected vitamin D metabolite is 25-hydroxyvitamin D₃ or 25-hydroxyvitamin D₂, respectively. As to Ground 5,

Petitioner challenges claims 10 and 11 as obvious over Voyksner in combination with Watson and Rainbow. Pet. 48–51.

According to Petitioner, although Voyksner discloses measurement of dihydroxyvitamin D₃ using LC/MS/MS, it does not disclose measurement of its metabolic precursor, 25-hydroxyvitamin D₃ or the related vitamin D₂ metabolite, 25-hydroxyvitamin D₂. Pet. 49 (citing Ex. 1008 ¶ 88). Relying on the testimony of Dr. Fitzgerald, Petitioner contends that, in light of Watson and Rainbow, one of ordinary skill in the art would have been motivated to use Voyksner’s LC/MS/MS methods to determine the amount of the substantially more abundant, and physiologically important, dihydroxy metabolites recited in claims 10 and 11. *Id.* at 49–51 (citing Ex. 1002 ¶¶ 19, 21–22, and 89–92). So motivated, Petitioner contends that the skilled artisan would have made “the small modification of the Voyksner method to measure another vitamin D metabolite, such as [the] 25-hydroxyvitamin D₃ or 25-hydroxyvitamin D₂” recited in claims 10 and 11, respectively. *Id.* at 51 (citing Ex. 1002 ¶¶ 91–92).

More particularly, Petitioner argues that in analyzing multiple metabolites using a similar technique, thermospray LC-MS, Watson measured various vitamin D compounds including 25-hydroxyvitamin D₃ and its substantially less abundant metabolite, 1,25-dihydroxyvitamin D₃. *Id.* at 50. Petitioner further states that “when analyzing multiple vitamin D metabolites” Watson maximized the current for detection of the protonated, dehydrated form of 1,25-dihydroxyvitamin D₃ “because the *in vivo* concentration of this molecule is normally three orders of magnitude less than that of 25(OH)D₃.” *Id.* (citing Ex. 1007, 7, Fig. 2; Ex. 1002 ¶ 89).

Petitioner also points out that “Rainbow shows that 25(OH)D₃ and 25(OH)D₂ can be measured simultaneously by LC-MS/MS, as exemplified by the standard curve shown for each analyte, as well as the metabolite profile showing measurement, by LC-MS/MS, of 25(OH)D₂ and 25(OH)D₃ from a single patient plasma sample.” *Id.* (citing Ex. 1008, 1; Ex. 1002 ¶ 90).

As summarized by Dr. Fitzgerald, “the structure of vitamin D metabolites are all quite similar” and “one of ordinary skill in the art, reading Watson and Rainbow, would have reasonably expected that Voyksner’s method used to measure dihydroxyvitamin D₃ would also have been suitable to measure other, more abundant vitamin D metabolites, such as 25-hydroxyvitamin D₃ (25(OH)D₃) and/or 25-hydroxyvitamin D₂ (25(OH)D₂)” which, the record indicates, were the most abundant and clinically relevant of the vitamin D metabolites. Ex. 1002 ¶¶ 89, 91; Pet. 51; Ex. 1049, 31:6–32:17, 38:13–39:13.

According to Dr. Fitzgerald, “[a] person of ordinary skill in the art would have been motivated to apply Voyksner’s method to measure another vitamin D metabolite, such as 25-hydroxyvitamin D₃ (claim 10) and/or 25-hydroxyvitamin D₂ (claim 11), in view of Watson’s and Rainbow’s teachings that various vitamin D metabolites can be measured using the same technique (Watson) and even simultaneously (Rainbow).” Ex. 1002 ¶ 92; *see also* Pet. 51.

Patent Owner opposes on the grounds that (1) Voyksner does not teach or render obvious the limitations of claim 1, from which claims 10 and 11 depend (PO Resp. 40), and (2) that Petitioner has failed to establish a motivation to combine Voyksner with Watson and/Rainbow with a reasonable expectation of success (*id.* at 4–5, 40–52; Sur-reply 16–21). In

section II.E, we determined that Petitioner established by a preponderance of the evidence that claim 1 was obvious over Voyksner in view of the knowledge of one of ordinary skill in the art. Because the parties rely on the same art and evidence for the limitations of claims 10 and 11 common to claim 1, we reach the same conclusion here.

With respect to motivation to combine/modify and reasonable expectation of success, Patent Owner argues, first, that Voyksner “is not a proper starting point” because it discloses “little more than the detection of an ion signal for ‘dihydroxyvitamin D₃’ in spiked rat plasma.” PO Resp. 42–43. For the reasons set forth with respect to Ground 1, above, we do not agree. *See also* Pet. 35–36, 46–47, 49–51 (citing, *e.g.*, Ex. 1002 ¶¶ 69–70, 87) (discussing reasons for combining Voyksner with the remaining the cited references); Reply 19–21 (further citing Ex. 1002 ¶¶ 21–23).

In short, the evidence of record strongly suggests that Voyksner’s “dihydroxyvitamin D₃” was a single vitamin D₃ metabolite and, more specifically, 1,25-hydroxyvitamin D₃. *See* section II.E.1, above. Because *in vivo* levels of 1,25-hydroxyvitamin D₃ were known to be “normally three orders of magnitude less than that of” the 25-hydroxyvitamin D₃ recited in claim 10, Voyksner’s method appears to exceed the sensitivity required for its detection. *See* Ex. 1002 ¶ 89; Ex. 1007, 7 (stating that the lower concentration of 1,25-hydroxyvitamin D₃, makes it “an important test of any proposed comprehensive analytical approach for [vitamin D metabolites]”); Ex. 1049, 39:8–13 (Dr. Sluss testifying that “the 25-hydroxy metabolites” are the most abundant vitamin D metabolites in healthy humans).

As further discussed with respect to Ground 1, one of ordinary skill in the art would have understood that Voyksner did not merely “detect[] an ion

signal,” as argued by Patent Owner, but took additional steps that entail correlating the known amount of vitamin D₃ in the sample with the amount of ions detected after mass spectrometry, and then quantifying the concentration of a vitamin D metabolite in the sample based on the detected ions. *See* PO Resp. 43; section II.2, above. We also agree with Petitioner that, in light of the general knowledge in the art, one of ordinary skill in the art would have found it obvious to apply Voyksner’s methods to quantify unknown amounts of vitamin D metabolites in a sample, and would have had a reasonable expectation of success in doing so. *See* section II.2, above. Accordingly, Voyksner provides a reasonable starting point for Ground 5.

Patent Owner also attacks the combination of Ground 5 references as “ill-suited” and directed to “different technical methods.” PO Resp. 43–46; Sur-reply 16–21. Patent Owner admits that “Watson discloses protonated and dehydrated ions of the vitamin D₃ metabolites 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃,” but argues that Watson’s thermospray process and “on-line detector” comprised “a completely different ionization technique” as compared to Voyksner’s off-axis nebulizer method. *Id.* at 43 (citing Ex. 1007, 4; Ex. 1004, 6–7; Ex. 2009 ¶ 138). Moreover, Patent Owner argues, Watson’s technique was “stale and obsolete” as of the publication of Voykner, and “not particularly good at making quantitative determinations of vitamin D metabolites.” *Id.* at 4, 43 (citing Ex. 1007, 8). We do not find Patent Owner’s arguments persuasive for the reasons set forth at pages 21–23 of the Reply, which we adopt. Nor do we agree with Patent Owner that Watson is “obsolete.” Rather, that during prosecution of the ’427 patent, the inventors submitted Watson to the Examiner indicating that one of ordinary skill in the art would have considered Watson relevant

to the claimed invention. *See* Ex. 1001 code (56), page 2; Ex. 1003, 68; *see also* Tr. 21:9–23 (noting that Ding,¹⁶ published in 2010, cites Watson as reference 17); Ex. 2021, 780, 788.

In short, we do not find the differences in the two mass spectrometry techniques particularly relevant, as Petitioner does not suggest that one of ordinary skill in the art would have physically combined the two methods, nor is it necessary to do so. As set forth in *Keller*: “The test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference; nor is it that the claimed invention must be expressly suggested in any one or all of the references. Rather, the test is what the combined teachings of the references would have suggested to those of ordinary skill in the art.” *In re Keller*, 642 F.2d 413, 425 (CCPA 1981) (citations omitted). As set forth in section E, above, Voyksner discloses a LC/MS/MS method suitable for determining the amount of a vitamin D metabolite—most likely, 1,25-hydroxyvitamin D₃. Because Watson discloses that an older, and according to Patent Owner, less capable, mass spectroscopy system can measure the amount of not only the 25-hydroxyvitamin D₃ recited in claim 10, but even the far less abundant 1,25-dihydroxyvitamin D₃, we conclude that one of ordinary skill in the art would have understood Voyksner’s LC/MS/MS system to be similarly capable. *See, e.g.*, Ex. 1007, Fig. 1 (detection of 480 pmol of vitamin D metabolites including 25-hydroxyvitamin D₃), Fig. 2 (detection of 0.6 pmol of 1,25-hydroxyvitamin D₃).

¹⁶ Ding et al., *Quantitative determination of vitamin D metabolites in plasma using UHPLC-MS/MS*, ANAL. BIOANAL. CHEM. 398:779–789 (2010). Ex. 2021.

Patent Owner argues that Rainbow fails to explicitly recite certain elements of claims 10 and 11, including protonated and dehydrated precursor ions, “one or more fragment ions,” and a correlation to the amount of vitamin D metabolite present in the sample. PO Resp. 44–45 (citing, e.g., Ex. 2009 ¶¶ 129–130). Although Patent Owner does not challenge Rainbow as lacking enablement, it further argues that Rainbow’s results and limited methods section are insufficient to “inform a POSITA about what adaptations a POSITA should make to Voyksner to meet all of the limitations of claims 10 and 11.” *Id.* at 45 (citing Ex. 2009 ¶ 130); Tr. 43:11–44:2.

Relying on the testimony of Dr. Fitzgerald, Petitioner responds that, although Rainbow uses protonated—but not dehydrated—precursor and precursor fragments, the use of protonated, dehydrated entities is disclosed in Voyksner and Watson, moreover, “selection of the precursor and fragment ions is a routine matter in developing MS/MS assays.” Reply 26 (citing Ex. 1002 ¶¶ 59, 90). In this respect, Dr. Fitzgerald testifies that:

The precursor and fragment ions formed are often simply determined by the model of the mass spectrometer used and/or the conditions for ionization. Considerations when selecting precursor and fragment ions included the relative abundance of the ions and the specificity of the ions for the analyte of interest (i.e., a peak not generated by molecules other than the analyte of interest). Kissmeyer EX1005, 10. The abundance of both the precursor and product ions would be optimized for maximum abundance to develop suitable precursor/product ion pairs for performing quantitative analysis. *Id.*

Ex. 1002 ¶ 59; *see also*; Tr. 29:20–30:2, 31:8–20, 44:3–15 (Patent Owner’s admission that there was nothing novel about using a protonated, dehydrated precursor ion). Considering the evidence of record, we find Petitioner’s argument persuasive.

We note that Rainbow describes calibration samples having known amounts of 25(OH)D₂ and 25(OH)D₃; generates calibration curves for quantitation of each metabolite by LC-MS/MS; and provides an ion chromatogram for a patient plasma sample, showing that a single assay produces fragment peaks for three hydroxyvitamin D metabolites: 25(OH)D₃, 25(OH)D₂, and 1,25(OH)D₂. Ex. 1008, 1 (Materials & Methods, Results); Ex. 1002 ¶ 51; *see also* Ex. 1008, 1 (Results). Rainbow, thus, demonstrates that a single LC-MS/MS methodology “can be used to routinely assay and quantify plasma levels of vitamin D2 & D3 metabolites.” Ex. 1008, 1 (Conclusions); Ex. 1002 ¶ 90. As such, we are persuaded that Rainbow relates the detected ions to the amount of vitamin D metabolite in said sample, including where the vitamin D metabolites are those recited in claims 10 and 11.

With respect to Patent Owner’s argument that Rainbow provides insufficient guidance to permit one of ordinary skill in the art to modify Voyksner to meet all of the limitations of claims 10 and 11, we note that Rainbow provides information on its purification method and equipment, the model and configuration of mass spectroscopy equipment (Applied Biosystems/MDS Sciex API 3000™ Triple Quadrupole Mass Spectrometer fitted with a TurboIonspray® ion source), materials and methods used for calibration curves, and information regarding data capture and analysis. *See* section II.D.6, above.

In light of the above, the high level of ordinary skill in the art, and the success of Voyksner, Watson, and Rainbow in assaying various vitamin D metabolites using related methodologies, Petitioner has established that claims 10 and 11 would have been obvious. Patent Owner does not persuade

us that one of ordinary skill in the art would not have known how to account for equipment differences between the cited references. *See* Sur-reply 18–19. Nor does Patent Owner persuade us that one of ordinary skill in the art would have needed more than the common knowledge in the art to modify Voyksner in light of Rainbow and/or Watson to arrive at the claimed invention. *See* Reply 19–27; Ex. 1002 ¶¶ 88–92.

III. CONCLUSION¹⁷

On the present record, Petitioner has established by a preponderance of the evidence that cited references would have taught or suggested each element of claims 1–11 of the '427 patent, and has set forth a sufficient rationale for why a person of ordinary skill would have been motivated to combine these teachings and suggestions to arrive at the invention recited in those claims. Accordingly, and for the foregoing reasons, Petitioner has shown by a preponderance of the evidence that claims 1–11 of the '427 patent are unpatentable, as summarized in the following table:

¹⁷ Should Patent Owner wish to pursue amendment of the challenged claims in a reissue or reexamination proceeding subsequent to the issuance of this Decision, we draw Patent Owner's attention to the April 2019 *Notice Regarding Options for Amendments by Patent Owner Through Reissue or Reexamination During a Pending AIA Trial Proceeding*. *See* 84 Fed. Reg. 16,654 (Apr. 22, 2019). If Patent Owner chooses to file a reissue application or a request for reexamination of the challenged patent, we remind Patent Owner of its continuing obligation to notify the Board of any such related matters in updated mandatory notices. *See* 37 C.F.R. §§ 42.8(a)(3), (b)(2).

Claims	35 U.S.C §	Reference(s)/ Basis	Claims Shown Unpatentable	Claims Not Shown Unpatentable
1–5	§ 103(a)	Voyksner	1–5	
6, 7	§ 103(a)	Voyksner, Kissmeyer	6, 7	
8	§ 103(a)	Voyksner, Kamao	8	
9	§ 103(a)	Voyksner, Watson, Vogeser	9	
10, 11	§ 103(a)	Voyksner, Watson, Rainbow	10, 11	
Overall Outcome			1–11	

IV. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that claims 1–11 of U.S. Patent No. 8,101,427 B2 are determined to be unpatentable; and

FURTHER ORDERED that, because this is a final written decision, parties to the proceeding seeking judicial review of the decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

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