Claims

1. An analytical method for analyzing the presence and quantity of impurities present in a preparation of dianhydrogalactitol comprises the steps of:

(a) analyzing a preparation of dianhydrogalactitol by subjecting the preparation to high performance liquid chromatography using elution with a mobile phase gradient to separate dianhydrogalactitol from dulcitol and other contaminants of the preparation; and

(b) determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself, wherein the compounds other than dianhydrogalactitol itself are at least one of: (1) dulcitol; (2) an impurity other than dulcitol; and (3) a degradation product of dianhydrogalactitol.

2. The analytical method of claim 1 wherein elution is with a gradient of NaOH from 2.5 mM to 0.1 mM.

3. The analytical method of claim 2 wherein elution is with a gradient of NaOH from 1.5 mM to 0.1 mM.

4. The analytical method of claim 3 wherein elution is with a gradient of NaOH from 1 mM to 0.1 mM.

5. The analytical method of claim 1 wherein elution is with a gradient of a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is a gradient that is from 2.5 mM to 0.1 mM.

6. The analytical method of claim 1 wherein elution is with a gradient of a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is a gradient that is from 1.5 mM to 0.1 mM.

7. The analytical method of claim 1 wherein elution is with a gradient of a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is a gradient that is from 1 mM to 0.1 mM.

8. The analytical method of claim 5, 6, or 7 wherein the proportion of ammonium hydroxide and the volatile ammonium salt selected from the group consisting of

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ammonium formate and ammonium acetate is varied from 100:1 at the beginning of elution to 1:100 at the end of elution.

9. The analytical method of claim 1 wherein the step of determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself is performed by evaporative light scattering detection.

10. The analytical method of claim 9 wherein the evaporative light scattering detection comprises post-column addition of a volatile solvent to enhance evaporation of the 100% aqueous mobile phase.

11. The analytical method of claim 10 wherein the volatile solvent is selected from the group consisting of methanol, ethanol, isopropanol, and acetonitrile.

12. The analytical method of claim 1 wherein an electrospray tandem mass spectrometer is installed and connected on-line to an HPLC system with ELSD.

13. The analytical method of claim 12 wherein tandem mass spectral data providing chemical information for each of the impurities and degradation products that may be present in a preparation of dianhydrogalactitol is collected.

14. The analytical method of claim 13 wherein the mass spectroscopy in tandem with HPLC provides molecular ion information and possible chemical structures having a molecular weight consistent with the molecular ion information for each of the observed impurities and degradation products.

15. The analytical method of claim 13 wherein at least one impurity or degradation product is identified by separation by column chromatography followed by at least one purification procedure to yield a solid unknown sample.

16. The analytical method of claim 15 wherein the solid unknown sample is characterized for identification by at least one standard analytical procedure selected from the group consisting of nuclear magnetic resonance (NMR), mass spectroscopy (MS), Fourier transform infrared spectroscopy (FT-IR), elemental analysis, determination of purity by HPLC, and determination of water content by the Karl Fischer titration method.

17. The analytical method of claim 1 further comprising the step of performing preparative HPLC collection of at least one specific substance peak present in a preparation of dianhydrogalactitol.

18. The analytical method of claim 17 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is an impurity.

19. The analytical method of claim 17 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is a degradation product.

20. An analytical method comprising:

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(a) analyzing a preparation of dianhydrogalactitol by subjecting the preparation to high performance liquid chromatography using elution with an isocratic mobile phase to separate dianhydrogalactitol from dulcitol and other contaminants of the preparation; and

(b) determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself, wherein the compounds other than dianhydrogalactitol itself are at least one of: (1) dulcitol; (2) an impurity other than dulcitol; and (3) a degradation product of dianhydrogalactitol.

21. The analytical method of claim 20 wherein the isocratic mobile phase is NaOH, and the concentration of NaOH is from 5 mM to 0.1 mM.

22. The analytical method of claim 21 wherein the concentration of NaOH is from 2.5 mM to 0.1 mM.

23. The analytical method of claim 22 wherein the concentration of NaOH is 1 mM.

24. The analytical method of claim 20 wherein the isocratic mobile phase is a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is from 5 mM to 0.1 mM.

25. The analytical method of claim 24 wherein the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is from 2.5 mM to 0.1 mM.

26. The analytical method of claim 25 wherein the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is 1 mM.

27. The analytical method of claim 26 wherein the proportion of ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is 50:50.

28. The analytical method of claim 20 wherein the step of determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself is performed by evaporative light scattering detection.

29. The analytical method of claim 28 wherein the evaporative light scattering detection comprises post-column addition of a volatile solvent to enhance evaporation of the 100% aqueous mobile phase.

30. The analytical method of claim 29 wherein the volatile solvent is selected from the group consisting of methanol, ethanol, isopropanol, and acetonitrile.

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31. The analytical method of claim 20 wherein an electrospray tandem mass spectrometer is installed and connected on-line to an HPLC system with ELSD.

32. The analytical method of claim 31 wherein tandem mass spectral data providing structural information for each of the impurities that may be present in a preparation of dianhydrogalactitol is collected.

33. The analytical method of claim 32 wherein the mass spectroscopy in tandem with HLPC provides molecular ion information and possible chemical structures having a molecular weight consistent with the molecular ion information for each of the observed impurities and degradation products.

34. The analytical method of claim 32 wherein at least one impurity or degradation product is identified by separation by column chromatography followed by at least one purification procedure to yield a solid unknown sample.

35. The analytical method of claim 34 wherein the solid unknown sample is characterized for identification by at least one standard analytical procedure selected from the group consisting of nuclear magnetic resonance (NMR), mass spectroscopy (MS), Fourier transform infrared spectroscopy (FT-IR), elemental analysis, determination of purity by HPLC, and determination of water content by the Karl Fischer titration method.

36. The analytical method of claim 20 further comprising the step of performing preparative HPLC collection of at least one specific substance peak present in a preparation of dianhydrogalactitol.

37. The analytical method of claim 36 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is an impurity.

38. The analytical method of claim 36 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is a degradation product.

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Claims

1. An analytical method for analyzing the presence and quantity of impurities present in a preparation of dianhydrogalactitol, wherein the impurities include dulcitol, comprising the steps of:

(a) analyzing a preparation of dianhydrogalactitol by subjecting the preparation to high performance liquid chromatography using elution with a mobile phase gradient to separate dianhydrogalactitol from dulcitol and other contaminants of the preparation; and

(b) determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself, wherein the compounds other than dianhydrogalactitol itself include dulcitol and optionally include (1) an impurity other than dulcitol; and (2) a degradation product of dianhydrogalactitol.

2. The analytical method of claim 1 wherein elution is with a gradient of NaOH from 2.5 mM to 0.1 mM.

3. The analytical method of claim 2 wherein elution is with a gradient of NaOH from 1.5 mM to 0.1 mM.

4. The analytical method of claim 3 wherein elution is with a gradient of NaOH from 1 mM to 0.1 mM.

5. The analytical method of claim 1 wherein elution is with a gradient of a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is a gradient that is from 2.5 mM to 0.1 mM.

6. The analytical method of claim 1 wherein elution is with a gradient of a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is a gradient that is from 1.5 mM to 0.1 mM.

7. The analytical method of claim 1 wherein elution is with a gradient of a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is a gradient that is from 1 mM to 0.1 mM.

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8. The analytical method of claim 5, 6, or 7 wherein the proportion of ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is varied from 100:1 at the beginning of elution to 1:100 at the end of elution.

9. The analytical method of claim 1 wherein the step of determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself is performed by evaporative light scattering detection.

10. The analytical method of claim 9 wherein the evaporative light scattering detection comprises post-column addition of a volatile solvent to enhance evaporation of the 100% aqueous mobile phase.

11. The analytical method of claim 10 wherein the volatile solvent is selected from the group consisting of methanol, ethanol, isopropanol, and acetonitrile.

12. The analytical method of claim 1 wherein an electrospray tandem mass spectrometer is installed and connected on-line to an HPLC system with ELSD.

13. The analytical method of claim 12 wherein tandem mass spectral data providing chemical information for each of the impurities and degradation products that may be present in a preparation of dianhydrogalactitol is collected.

14. The analytical method of claim 13 wherein the mass spectroscopy in tandem with HPLC provides molecular ion information and possible chemical structures having a molecular weight consistent with the molecular ion information for each of the observed impurities and degradation products.

15. The analytical method of claim 13 wherein at least one impurity or degradation product is identified by separation by column chromatography followed by at least one purification procedure to yield a solid unknown sample.

16. The analytical method of claim 15 wherein the solid unknown sample is characterized for identification by at least one standard analytical procedure selected from the group consisting of nuclear magnetic resonance (NMR), mass spectroscopy (MS), Fourier transform infrared spectroscopy (FT-IR), elemental analysis, determination of purity by HPLC, and determination of water content by the Karl Fischer titration method.

17. The analytical method of claim 1 further comprising the step of performing preparative HPLC collection of at least one specific substance peak present in a preparation of dianhydrogalactitol.

18. The analytical method of claim 17 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is an impurity.

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19. The analytical method of claim 17 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is a degradation product.

20. An analytical method for analyzing the presence and quantity of impurities present in a preparation of dianhydrogalactitol, wherein the impurities include dulcitol, comprising the steps of:

(a) analyzing a preparation of dianhydrogalactitol by subjecting the preparation to high performance liquid chromatography using elution with an isocratic mobile phase to separate dianhydrogalactitol from dulcitol and other contaminants of the preparation; and

(b) determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself, wherein the compounds other than dianhydrogalactitol itself include dulcitol and optionally include (1) an impurity other than dulcitol; and (2) a degradation product of dianhydrogalactitol.

21. The analytical method of claim 20 wherein the isocratic mobile phase is NaOH, and the concentration of NaOH is from 5 mM to 0.1 mM.

22. The analytical method of claim 21 wherein the concentration of NaOH is from 2.5 mM to 0.1 mM.

23. The analytical method of claim 22 wherein the concentration of NaOH is 1 mM.

24. The analytical method of claim 20 wherein the isocratic mobile phase is a combination of ammonium hydroxide and a volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate and the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is from 5 mM to 0.1 mM.

25. The analytical method of claim 24 wherein the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is from 2.5 mM to 0.1 mM.

26. The analytical method of claim 25 wherein the total concentration of the ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is 1 mM.

27. The analytical method of claim 26 wherein the proportion of ammonium hydroxide and the volatile ammonium salt selected from the group consisting of ammonium formate and ammonium acetate is 50:50.

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28. The analytical method of claim 20 wherein the step of determining the relative concentration of one or more peaks resolved by high performance liquid chromatography that represent compounds other than dianhydrogalactitol itself is performed by evaporative light scattering detection.

29. The analytical method of claim 28 wherein the evaporative light scattering detection comprises post-column addition of a volatile solvent to enhance evaporation of the 100% aqueous mobile phase.

30. The analytical method of claim 29 wherein the volatile solvent is selected from the group consisting of methanol, ethanol, isopropanol, and acetonitrile.

31. The analytical method of claim 20 wherein an electrospray tandem mass spectrometer is installed and connected on-line to an HPLC system with ELSD.

32. The analytical method of claim 31 wherein tandem mass spectral data providing structural information for each of the impurities that may be present in a preparation of dianhydrogalactitol is collected.

33. The analytical method of claim 32 wherein the mass spectroscopy in tandem with HLPC provides molecular ion information and possible chemical structures having a molecular weight consistent with the molecular ion information for each of the observed impurities and degradation products.

34. The analytical method of claim 32 wherein at least one impurity or degradation product is identified by separation by column chromatography followed by at least one purification procedure to yield a solid unknown sample.

35. The analytical method of claim 34 wherein the solid unknown sample is characterized for identification by at least one standard analytical procedure selected from the group consisting of nuclear magnetic resonance (NMR), mass spectroscopy (MS), Fourier transform infrared spectroscopy (FT-IR), elemental analysis, determination of purity by HPLC, and determination of water content by the Karl Fischer titration method.

36. The analytical method of claim 20 further comprising the step of performing preparative HPLC collection of at least one specific substance peak present in a preparation of dianhydrogalactitol.

37. The analytical method of claim 36 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is an impurity.

38. The analytical method of claim 36 wherein the at least one substance peak present in the preparation of dianhydrogalactitol is a degradation product.

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