Natural Product Preparative HPLC Purification from Complex Crude Extraction Mixtures by Granular Bonded and Unbonded Silica Gel

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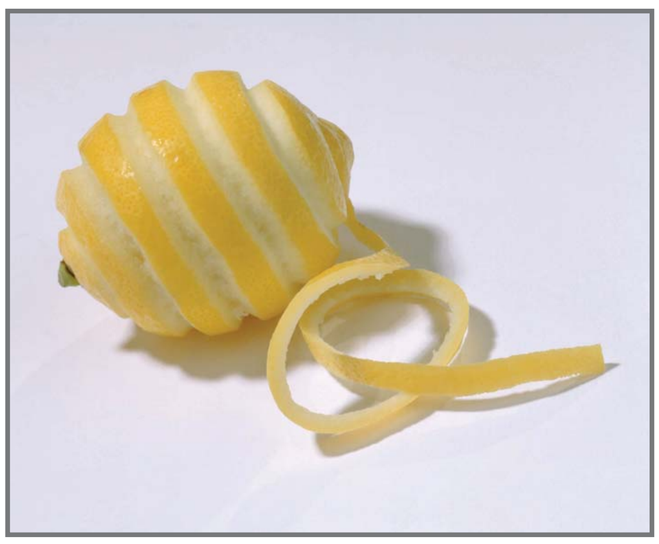
Abstract

The reliable enrichment, analysis and purification of low abundance natural product constituents from their complex crude extraction mixtures by liquid chromatography remains a key challenge in the pharmaceutical, food and flavors and natural products fields. Davisil® granular silica can bring substantial economy to the purification and enrichment of natural products and is shown to resolve difficult mixtures well at a large scale via normal and reversed phase HPLC. Natural products such as terpenes and terpenoids are a major factor in pharmaceuticals (paclitaxel), food and flavorings (citrus flavor) and microbiocidals (natural fungicides). Purification of terpenes and terpenoids is demonstrated on Davisil® C18 bonded silica for Paclitaxel extracted from Yew bark and on Davisil® normal phase silica for purification of the flavor and fragrance components, Limonene and α-Terpinene, from the essential oil of lemon. Vitamin E, Isomeric normal phase HPLC purification was developed that utilizes the substantial economy Davisil® offers through a two step purification strategy. The adverse affects of the γ-isomer on the chromatography of the desired β-isomer under practical overload conditions were minimized by the two-step process resulting in a product that was greater than 93% pure.

Introduction



The role of the stationary phase in preparative chromatographic separations is vital for the fine resolution of difficult mixtures. The enrichment and separation of low abundance natural product constituents from their complex crude extraction mixtures by Normal Phase and Reversed Phase HPLC requires a high degree of resolution, but often requires the benefit of substantial economy delivered by granular silica. Davisil® granular silica, being less expensive than spherical silica, brings substantial economy to the purification process while conquering the challenges required to remove slight impurities, isomeric mixtures and removal of analogous molecules in the pharmaceutical, food and flavors and natural products fields. Natural products such as Terpenes and Terpenoids are a major factor in pharmaceuticals (Paclitaxel), food and flavorings (citrus flavor) and microbiocidals (natural fungicides). Paclitaxel, or Taxol by brand name, is an anti-cancer chemotherapy drug used to treat breast, ovarian, lung, bladder, prostate, melanoma, esophageal, as well as other types of solid tumor cancers and has also been used to treat Kaposi's sarcoma. Removing impurities from Paclitaxel is particularly challenging, since the molecular structures in the extracted mixture are very similar. Effective enrichment of the moderately polar target molecule, Paclitaxel and the closely related compound Cephalomannine was performed with Davisil® C18 silica. The majority of crude impurities were easily removed from the extraction sample utilizing the superior bonding technology and purity of Davisil® C18 silica to effectively retain and enrich the moderately polar target molecules. Grace Davisil® C18 silica purified Taxol from 90% of the yew bark extract impurities. The purification of the flavor and fragrance components, α-Terpinene and Limonene from the essential oil of lemon was performed on Davisil® chromatographic silica. Terpenes are naturally occurring compounds mainly found in plants as essential oil constituents whose carbon skeletons are composed exclusively of isoprene C5 units ($CH_2=C(CH_3)-CH=CH_2$). The target Terpenes extracted from the essential of lemon were effectively purified by normal phase LPHPLC on Davisil® silica. Isomeric separations of natural products is demonstrated on Davisil® silica. Separation of isomers in natural products requires superior performance characteristics at a relatively large scale when using granular silica while maintaining a high economic value for products such as Vitamin E, with a low added value. Davisil® granular silica, being less expensive than spherical silica, delivers substantial economy to the isomeric purification, while allowing the fine resolution of the β-isomer of Tocopherol from a mixture of α, β, γ and δ isomers.

Experimental

Citrus Flavor Purification

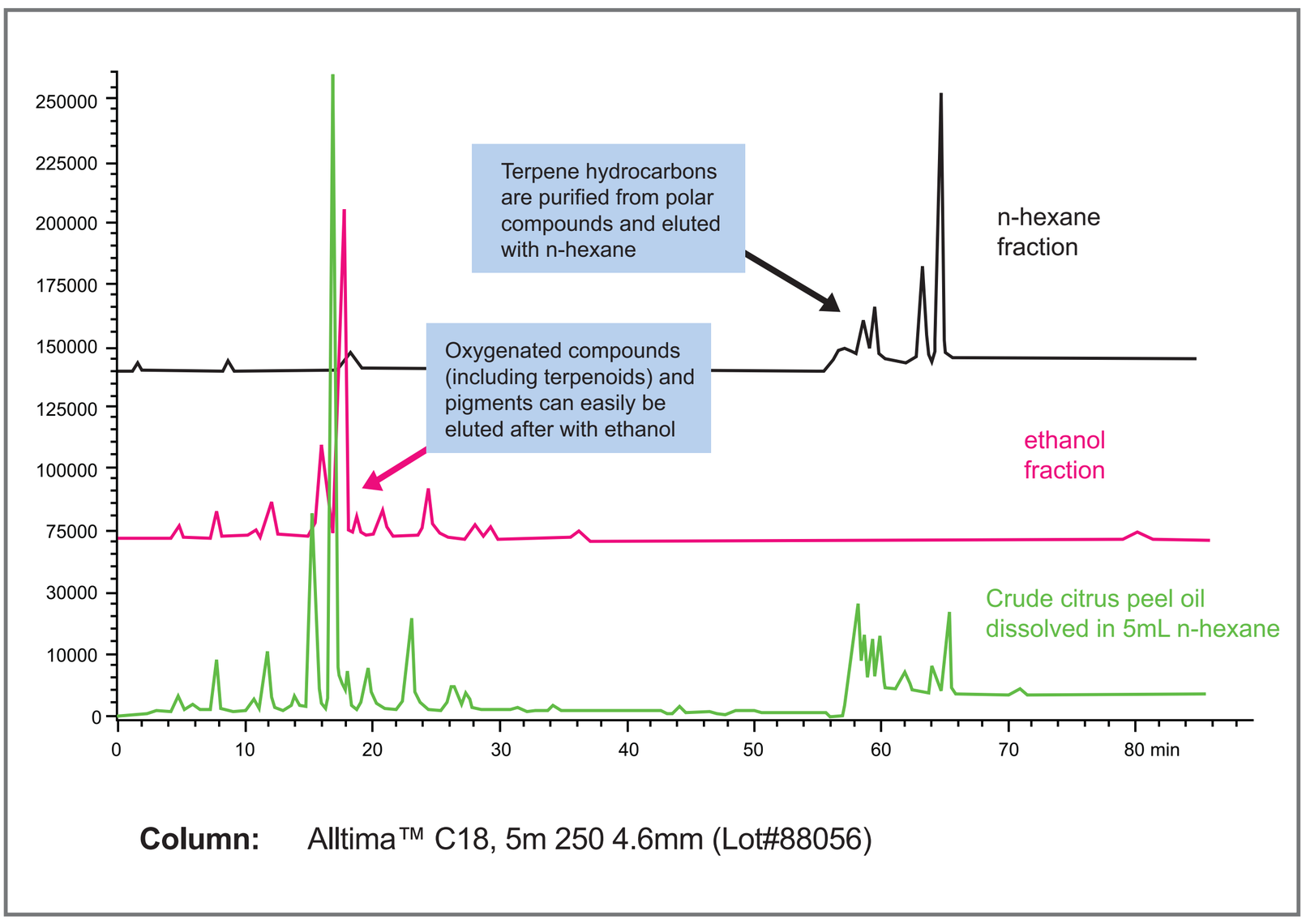
Extraction

- 180g of fresh citrus peel extracted twice with sonication
- Extract was impregnated for 24 hours with 450ml petroleum ether (30-60°C)
- Crude citrus oil obtained after concentration under vacuum and dissolved with ethanol, frozen at 4°C for 48 hours and centrifugally separated and filtered

Purification

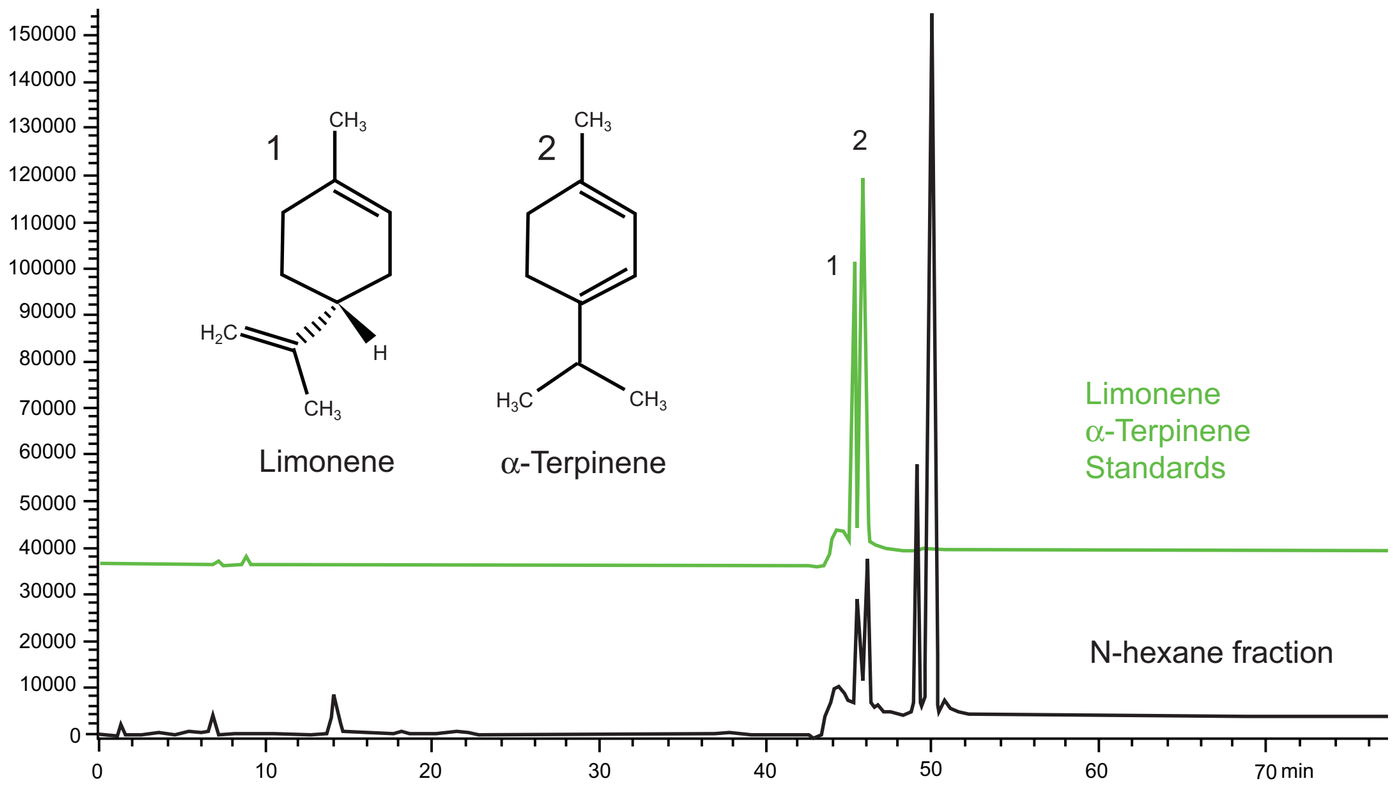
- 80g of Grace Davisil® LC60Å 40-63 silica gel slurried with 120ml n-hexane in 25mm i.d. x300mm glass column
- Crude citrus oil in 5ml n-hexane injected onto grace Davisil® silica gel column
- N-hexane wash followed by ethanol wash
- Two fractions collected
- Fractions analyzed by RP-HPLC
- Standard comparison with n-hexane fraction determined components

Figure 1. RP-HPLC Analysis of Collected Fractions



Column: Alltima™ C18, 5m 250 4.6mm (Lot#88056)

Figure 2. Identification of Terpene hydrocarbons in enriched n-Hexane fraction



Column: Alltima™ C18, 5µm 250 4.6mm(Lot# 88056)
Mobile Phase: **A:** water; **B:** methanol
Flow Rate: 1.0mL/min
Detection: UV@254nm
Column Temp: 25°C

Gradient:

Time:	0	40	40	60
% B:	70	70	100	100

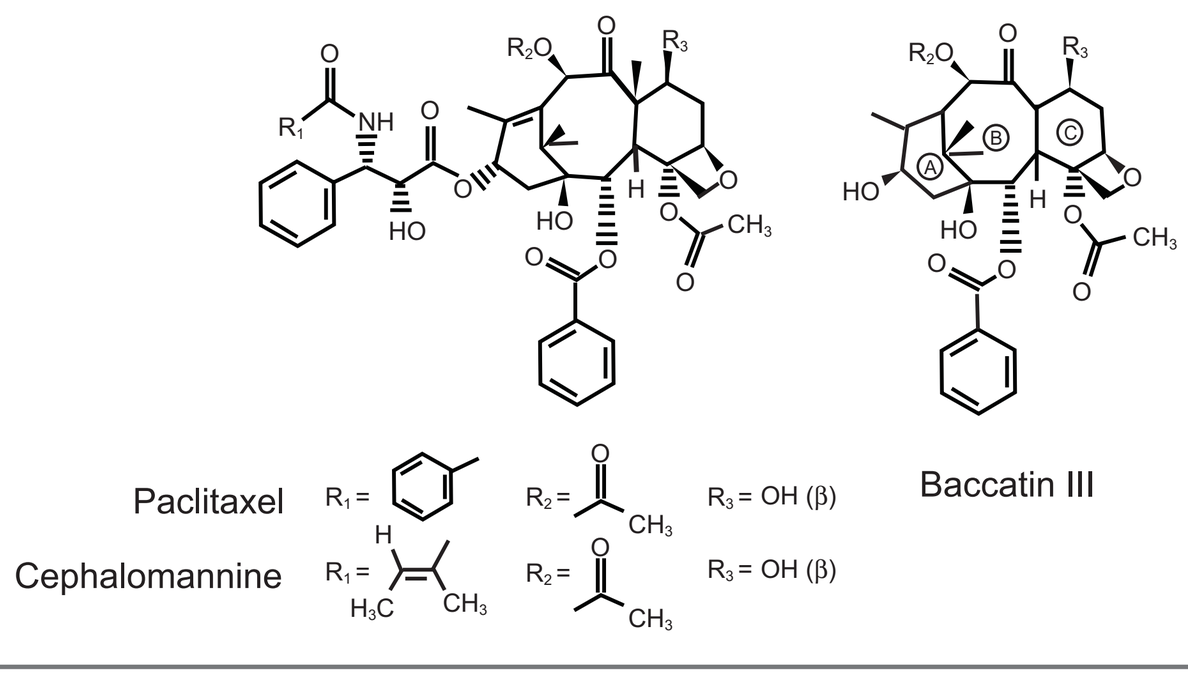
Results

Davisil® silica gel was used to effectively purify limonene and terpinene from the crude citrus oil extraction mixture. The effective retention of the oxygenated compounds and pigments allowed the target citrus flavor compounds, terpinene and limonene, to be easily eluted in the n-hexane fraction, purifying them from the crude material. Identification of the enriched material was easily performed on the high resolution, Alltima™ C18 analytical column. The polar compounds and pigments, including the terpenoid analogs, can be eluted with an ethanol fraction. Davisil® silica gel is an effective tool for simple separation and enrichment of polar and non-polar citrus flavor components from crude extraction mixtures with it's high purity, reproducibility and excellent resolution.

Removal of Polar Impurities From Paclitaxel Extracted From Yew Bark Powder Using Davisil® C18 Silica

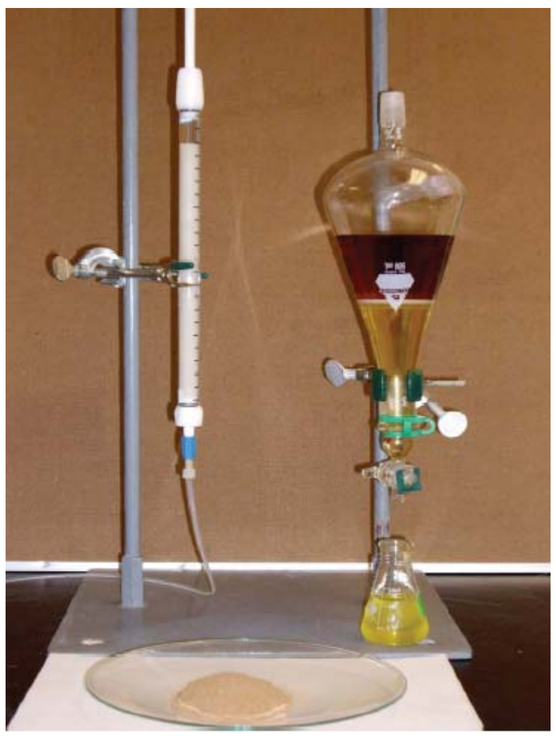
Removing impurities from paclitaxel is particularly challenging, since the molecular structures in the extracted mixture are very similar

The crude solvent extract contains paclitaxel and large quantities of highly polar taxane analogous impurities



Extraction

- 10 grams of the powdered yew bark
- 500ml of Methanol
- Roll for 12 hours at room temperature
- 400 ml yew bark extract concentrated to 200ml



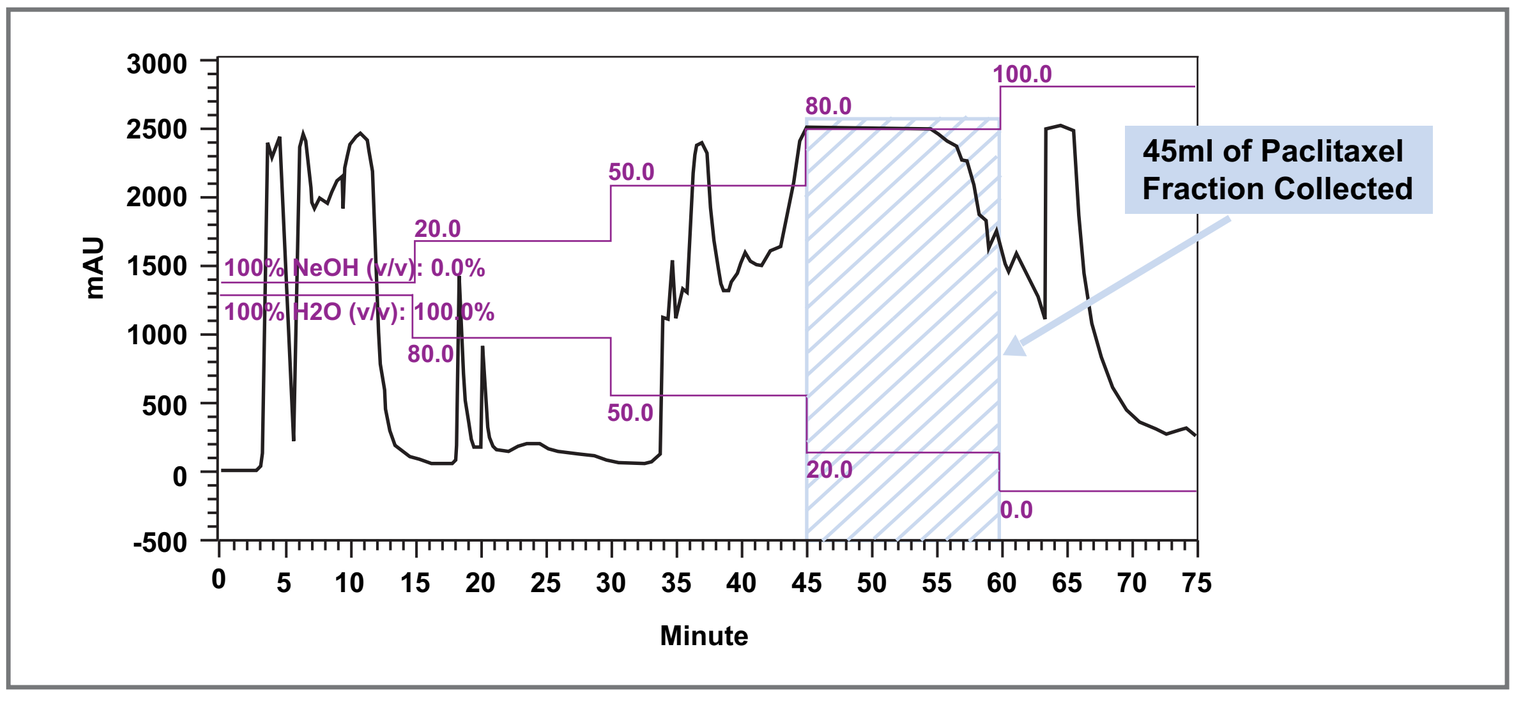
Liquid-liquid extraction

- 90ml of the concentrated extract with 100ml methylene chloride & 100 ml water
- Mix for 2 hours then equilibrate mixture in the separatory funnel for 12 hours
- Extracted solvent concentrated to 2ml injection sample

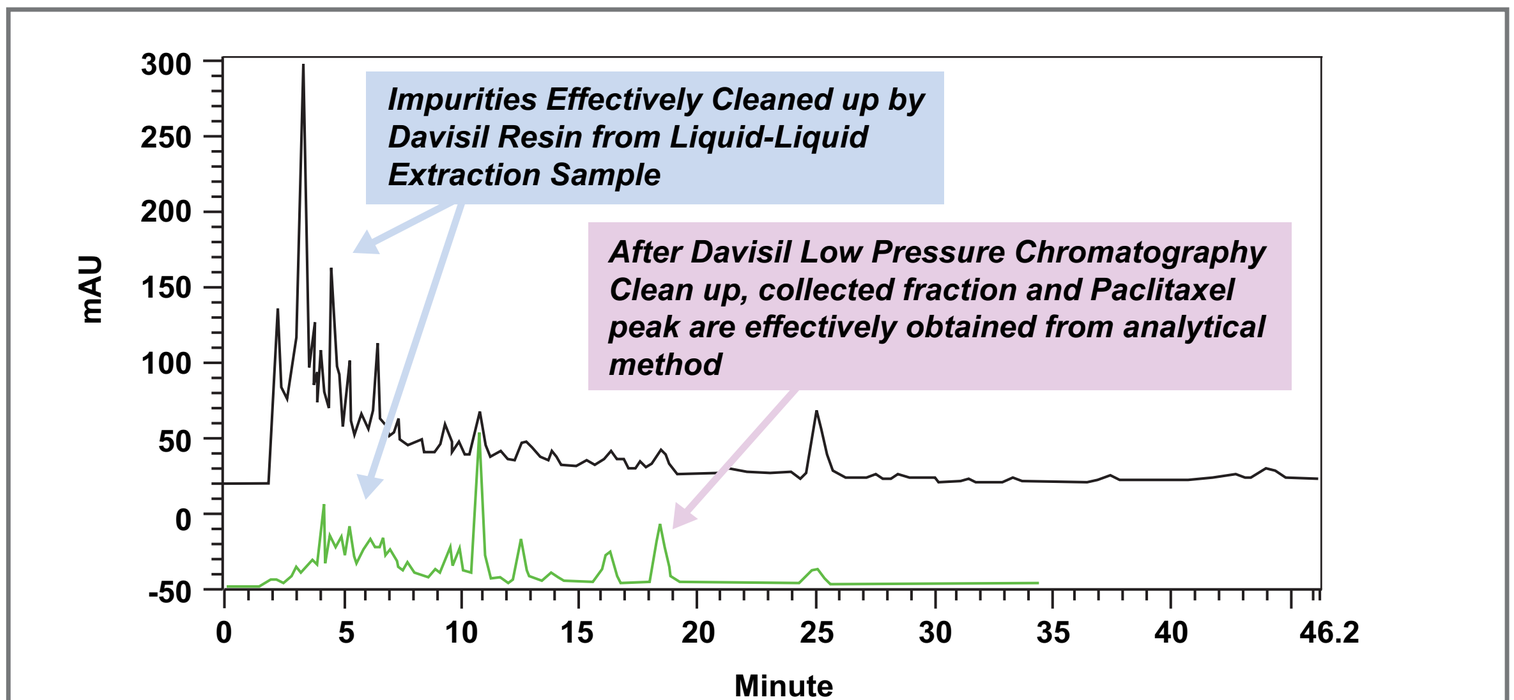
Purification

- 10 grams of 633N C18 slurried with 20ml of Isopropanol
- Slurry used to pack a 10 mm i.d.x200mm length column
- 80/20 Methanol/Water used to elute the target compound
- Fraction analyzed with a Grace Denali® C18 analytical column
- Taxol identified with the standard reference (Sigma Aldrich)

Low Pressure Preparative Chromatography with Grace Davisil® 633N C18 (10g) 10 mm i.d.x200mm Column



Low Pressure Chromatography Collected Fraction Analysis by Grace Vydac® 238DE54



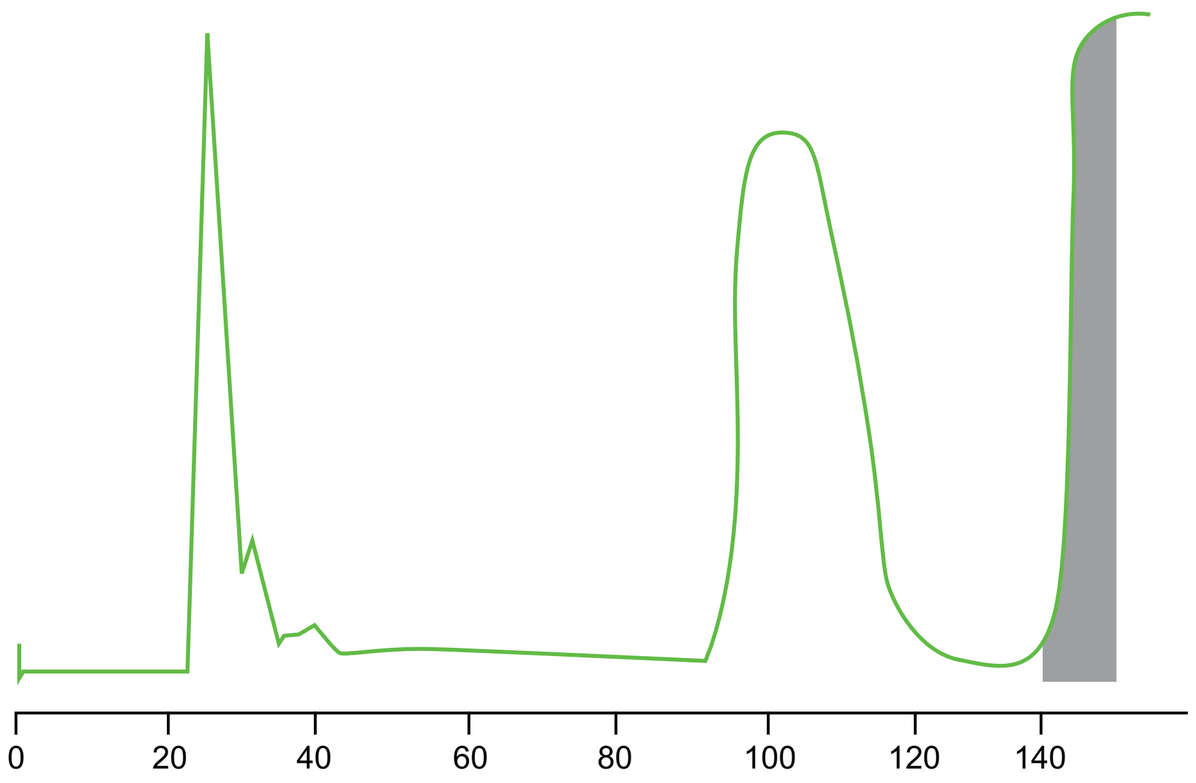
Results

- Grace Davisil® C18 silica effectively purified Taxol from 90% of yew bark extract impurities
- Grace Vydac® 238DE54 analytical column resolved the Taxol from closely eluting impurities for standard identification
- Davisil® C18 column utilized dispersive interactions and semi-polar interactions through crude separation by reversed phase
- Superior bonding technology and purity of Davisil® C18 silica effectively retained and enriched the moderately polar target molecule
- The majority of crude impurities were easily removed from the extraction sample
- The Denali® C18 analytical column was used for simple identification of the peaks of interest utilizing high retention, high efficiency and excellent peak symmetry
- Isolation of natural products such as paclitaxel can be greatly improved by crude separation on Davisil® C18 high purity media

Vitamin E Purification

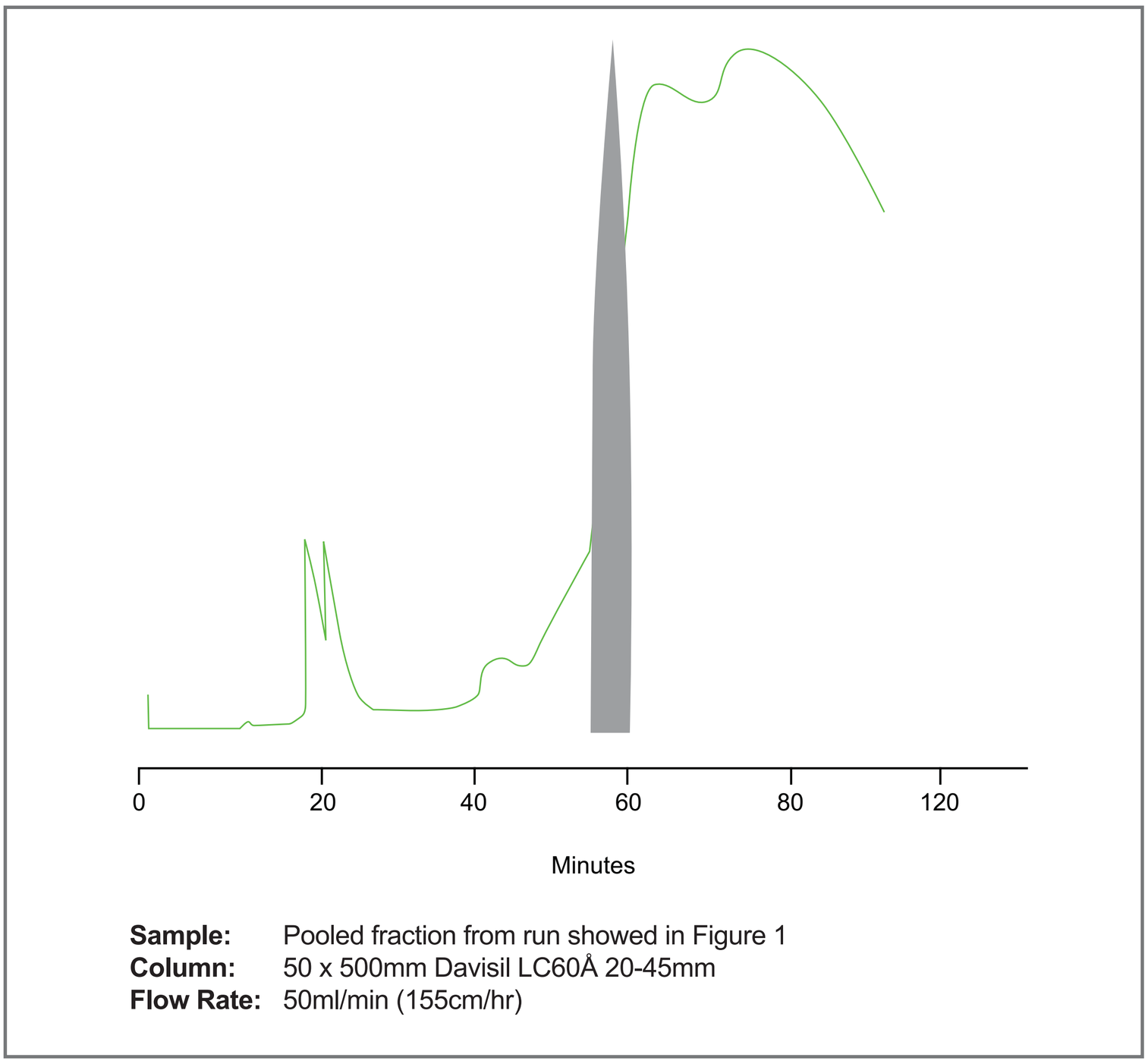
Loading studies on a 50mm diameter column with Davisil® silica LC60A 20-45µm, showed that in practical overload conditions, the large amount of the γ-isomer adversely affected the chromatography of the desired β-isomer. Therefore, a two step strategy was developed, in which a 200mm diameter column was first used to produce a pool highly enriched in b-isomer **(Figure 1)**, which was then rechromatographed on the 50mm column (**Figure 2**), resulting in a product which was more than 93% pure.

Figure 1. First step separation of tocopherol β-isomer on Davisil LC60Å 20-45mm Column. Shaded area indicates collected fraction



Sample: 250g tocopherol isomer mixture in 1000ml hexane
Column: 200 x 500mm Davisil® LC60A 20-45mm
Mobile Phase: hexane/2.5% THF
Flow Rate: 600ml/min (115cm/hr)

Figure 2. Rechromatography of β-isomer-enriched tocopherol from Figure 1. Shaded area indicates collected fractions



Sample: Pooled fraction from run showed in Figure 1
Column: 50 x 500mm Davisil LC60Å 20-45mm
Flow Rate: 50ml/min (155cm/hr)

Conclusions

- The enrichment and separation of a wide array of Natural products including Terpenes, Terpenoids, Paclitaxel and Vitamin E isomers are possible with reversed phase and normal phase Davisil Chromatographic Silica.
- Davisil granular silica, is less expensive than spherical silica, delivering substantial economy to preparative purifications, while allowing the fine resolution of complex mixtures.
- Effective enrichment of the moderately polar target molecules were easily performed with Davisil C18 silica.
- For complex isomers a two step strategy can be used with Davisil granular silica, in which one column can first be used to produce a pool highly enriched in one isomer, which can then be rechromatographed on a second column, to result in a highly purified final product without a significant media cost for low value products.