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PREPARATIVE LIQUID CHROMATOGRAPHY APPLIED TO DIFFICULT SEPARATIONS

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A serious drawback in high-pressure liquid chromatography (hplc) is the fact that although it is ideal for micro-scale separations, the maximum quantity that can be handled by a single injection is less than 100 mg. In the following we report the application of "prep-scale" hplc to selected difficult separations in the range of 10mg-15 g by usage of commercial and homemade columns[1].

The C_{18}-ketone[2] is a key intermediate in the synthesis of vitamin A, retinal, and various retinoids. The all-trans and 9-cis isomers, 1 and 2, are obtained from β-ionone by crossed aldol[3] or more recently by directed-aldol condensations[4]. Separation of 1 and 2, which is unattainable by classical methods, can be readily achieved by prep-lc (Fig. 1). Namely an 820 mg mixture of the two isomers can be separated in less than 20 minutes, the shaded areas in Fig. 1 corresponding to greater than 99% purity.

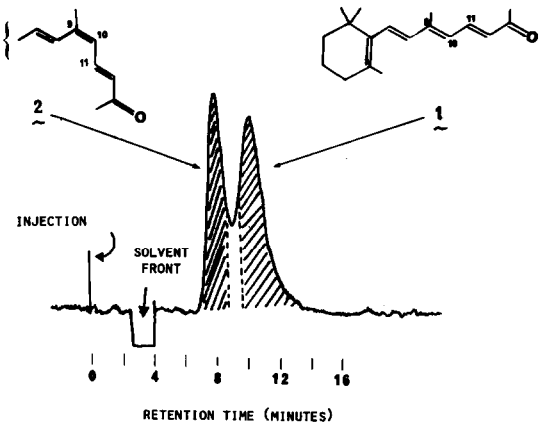


Fig. 1: Separation of an 820 mg mixture of all-trans 1 and 9-cis 2 C_{18}-ketones on a Waters Prep LC-500, Refractive Index Detector. 30 x 5 cm silica gel column, solvent = 11% ether in hexane; flow rate = 250 ml/min.

The chromophore of the visual pigment rhodopsin is 11-cis retinal. The syntheses of various model retinals[5] is essential in order to clarify the binding properties of the apoprotein opsin [5d,6] as well as other aspects of the complex nature of vision. A major obstacle is the difficulty in securing 10 mg quantities of a pure double bond isomer for ensuing binding studies because analytical hplc[5,7] can maximally handle only a few mg of these extremely labile (to heat, light, etc) compounds. The separation of retinals in the crucial 5-100 mg range, which are the average amounts obtained in synthesis, could be achieved, however, by modifying the instrument (Waters Prep-LC 500) so that it could be equipped with a uv detector since mixtures less than

100 mg cannot usually be seen by the refractive index detector. A 100% separation of a 32 mg mixture of all-trans retinal 3 and its 13-cis isomer 4 was achieved in less than 30 minutes (Fig. 2), and recovery of the isomers was quantitative. The inserts show analytical hplc traces of the samples before and after prep-lc.

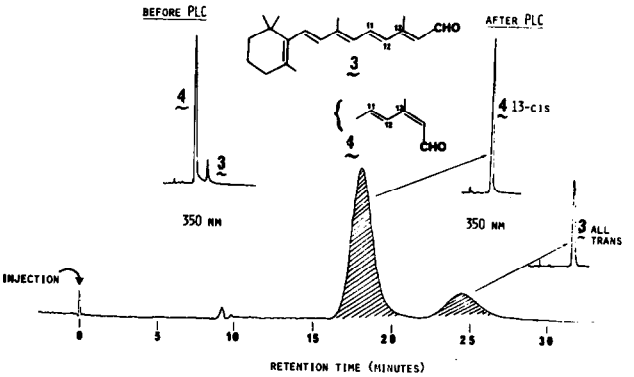


Fig. 2: Waters Prep LC-500 modified with a JASCO UVIDEC-100 variable wavelength uv detector λ = 254 A = 2.56, 30 x 5 cm silica gel column, solvent = 8% ether in hexane, flow rate = 200 ml/min (analytical conditions: 30 cm x 4 mm μ-porasil column, same solvent, flow rate = 2 ml/min, λ = 350 (∿ λ_{max} of retinals)

The separation of farnesol isomers represents a classical analytical problem. Separation of a 3 g mixture of all trans farnesol 5 and its 2Z isomer 6 by prep-lc was attained in less than 2 hours by a process employing one recycle of the first middle cut (Fig. 3). In the case of a mixture of all four farnesol double bond isomers, it was possible to separate the C-2 pairs, i.e., 2Z/6Z and 2Z/6E from 2E/6Z and 2E/6E, but the respective pairs could not be separated with the solvent system employed. Isomers around the trisubstituted double bonds could be readily characterized by the operation of the γ-effect[8] which led to an upfield shift of 7-8 ppm for olefinic methyl and allylic methylene carbons having cisoid γ-carbons as exemplified for 5 and 6 in Fig. 3.

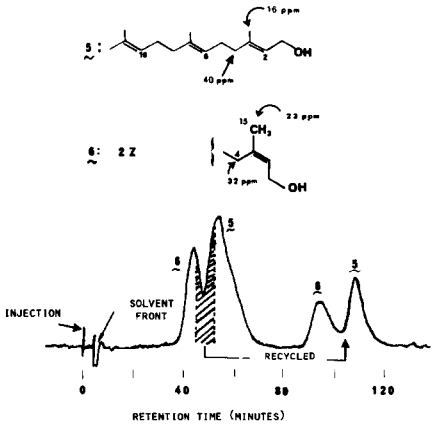
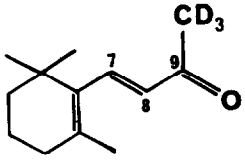


Fig 3: RI Detector, two-30 cm x 5 cm silica gel columns, 10% ether in hexane, flow rate = 250 ml/min.

A 1 g mixture of the cis and trans isomers, nerol and geraniol, has been similarly separated.

In contrast to the above cases which were difficult separations (i.e., low α values) unattainable via classical methods, prep-lc can be used for the isolation of more easily separated materials. The main advantage is that larger amounts can be separated in shorter periods of time, and consequently, the risk of sample deterioration is often minimized. A mixture containing 17.5 g of d_3-β-ionone 7, a key intermediate in the synthesis of deuterated vitamin A com-

pounds,[9] was separated from its main synthetic contaminant d_6-tert-alcohol 8 in less than 20



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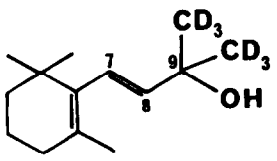

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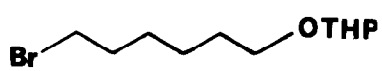

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minutes in a manner similar to those described above. Here, prep-lc replaced the conventional open column separation which would have taken approximately 24 working hours. Similarly 14 g of the bromotetrahydropyranyl ether 9 could be easily separated from numerous trace impurities (as seen by tlc) in less than 15 minutes using prep-lc instead of a time-consuming spinning band distillation.

The only commercially available cartridge (silica gel) for the Prep-LC 500 instrument gave poor separation (Fig. 4a) of the double bond isomers of the tetrahydropyranyl aldehydes 10, synthetic intermediates for an insect pheromone[10]. Since it is known that $AgNO_3$ coated silica gel greatly improves the separation of cis/trans isomers, a new cartridge was prepared by emptying a commercial one, impregnating its silica gel particles with 10% $AgNO_3$, refilling by dry-packing, and finally refitting the cartridge with the end frits. The success of this modification is illustrated in Fig. 4b in which a far better separation is achieved in significantly less time with shorter column length.

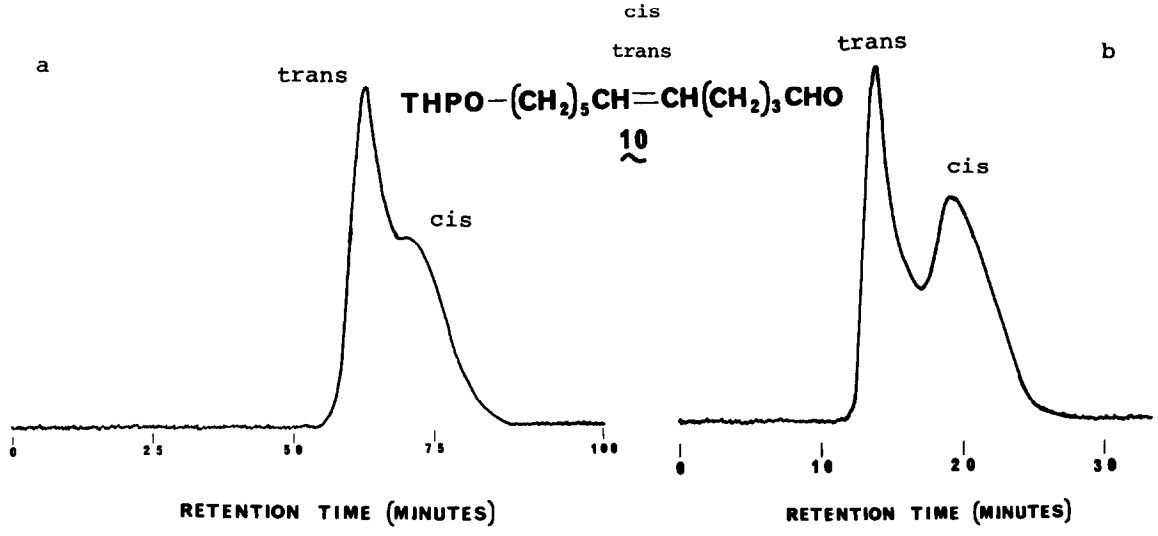

Fig. 4: Separation of a 450 mg mixture of cis and trans 10

RI Detector; solvent = 11% ether in hexane

a) two-30 cm x 5 cm silica gel columns; flow rate = 150 ml/min.

b) one-prepared 10% $AgNO_3$ on silica gel column; flow rate = 100 ml/min.

It is obvious that prep-lc could be carried out with the wide variety of packing materials available in analytical hplc. This would greatly increase the versatility and efficiency of numerous preparative separations, in particular those of polar compounds[11].

FOOTNOTES AND REFERENCES

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11) The studies were supported by NIH grant AI 10187.