

Chiral Separation of Abscisic Acid on Eurocel 01

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Abstract

Abscisic acid (ABA) occurs in two biologically active enantiomer forms in synthetic preparations. The ABA racemate was resolved here successfully using RP-HPLC and Eurocel 01, a cellulose based chiral stationary phase (CSP). Compared to existing methods using other CSPs, Eurocel 01 produces shorter retention times with comparable selectivity.

Introduction

As a plant growth regulator hormone, abscisic acid (ABA) is involved in a wide range of developmental and stress-related events¹⁻². While ABA can exist as two enantiomers³, when it is extracted from plant tissues only the (+) enantiomer of ABA (see Fig. 1) is recovered, indicating that this is the natural form of the hormone.⁴ The R-(-)-ABA form of the hormone is also biologically active⁵, making resolution of the commercially available ABA racemate necessary to investigate the pure enantiomers.

Enantioseparation of ABA is normally done using HPLC in either polar organic mode or reversed phase mode, due to the polarity and solution behavior of ABA. The versatile Eurocel 01 chiral stationary phase (CSP), based on a wide-pore silica matrix coated with a derivatized cellulose 3,5-dimethylphenylcarbamate chiral selector, was compared here to other CSPs using existing methods. The Eurocel CSP effects an impressive enantioselectivity by combining polar and π - π interactions with inclusion complexation.

Experimental Conditions

The analysis was performed on a KNAUER Smartline System, equipped with Pump 1000, Manager 5000 with degasser unit, Column Oven 4000, Autosampler 3900 and Detector 2500.

Column: Eurocel 01, 5 μ m, 250 x 4.6 mm
Detection: UV at 230 nm
Flow rate: 1 ml/min
Temperature: 25 °C
Injection volume: 5 μ l

Results

Our first attempt at resolving the ABA enantiomers involved using 100% methanol in polar organic mode. The eluent was adjusted with 0.1 % trifluoroacetic acid (TFA) due to the acidic character of the sample. Since this eluent did not produce any separation, we decided to lower the methanol content by adding water. Using methanol/water 80:20 (v/v) in the presence of 0.1 % TFA, the ABA sample coeluted as a split peak. Further analysis using a methanol/water mixture of 50:50 (v/v) and 0.1 % TFA produced a separation of the enantiomers within 15 min with a selectivity of 1.2 (Fig. 2). The results of the reversed phase mode separation were stable and could be reproduced without a loss of separation performance.

Conclusion

Compared to other chiral stationary phases used for enantioseparation of ABA (see Table 1), the Eurocel 01 phase has the shortest retention times with comparable selectivity. Besides its impressive chromatographic efficiency, Eurocel 01 can be applied in normal phase, reversed phase, as well as polar organic modes, giving the analyst more flexibility to tackle even the most challenging separation problems.

Table 1: Comparison of three chiral stationary phases

	Eluent	t_{R1} (ret. time)	k'_{1} (capacity)	t_{R2} (ret. time)	k'_{2} (capacity)	α (selectivity)
KNAUER Eurocel 01, 250 x 4.6 mm	Methanol: water (50:50) v/v, with 0.1% TFA	10.28	2.67	11.75	3.19	1.20
ChromTech Chiral AGP, 100 x 4 mm	75 mM sodium phosphate buffer, pH 5	15.15	2.16	17.80	2.70	1.25
Phenomenex Chirex 3020, 250 x 4.6 mm	hexane, 2- propanol and methanol (97:2:1) v/v	22.20	4.16	23.30	4.41	1.06

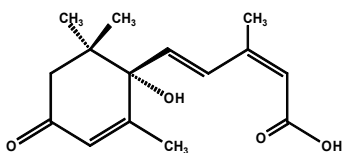


Figure 1: Chemical structure of the natural hormone S-(+)-abscisic acid

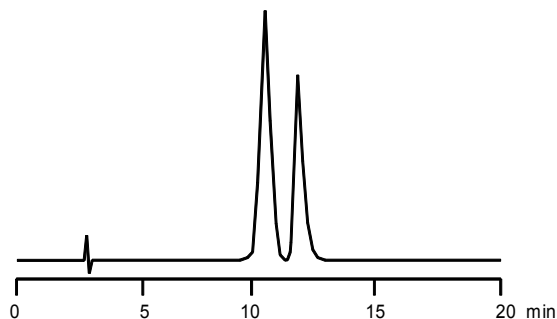


Figure 2: Separation of (±)-ABA using MeOH/water 50:50 (0.1 % TFA) on Eurocel 01

References

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