Flash Chromatography: Importance of Detection Method

Dual Detection Prevents Loss of Sample

Advantage Statement: Flash chromatography is a rapidly growing technique in the field of chromatography and is essentially a simplified, rapid form of preparative LC. As with traditional HPLC, the analysis of compounds that do not have a UV chromophore can cause problems due to their very low limit of detection. Post-column addition of a UV-active fragment, or fluorescing agent, is one option. However, a much simpler solution to this problem is the use of an evaporative light scattering detector (ELSD) coupled to the flash instrument.

This advantage note demonstrates the benefits of using a Varian 385-LC ELS detector alongside a 971-FP flash instrument for the analysis of a sample containing a mixture of UV and non-UV active compounds. The major advantage of dual detection is the ability to prevent loss of sample components that remain undetected by UV and are, therefore, not isolated by the fraction collector.

Software Control

The Varian 971-FP uses software to incorporate an ELSD as an alternative method of detection, and these two instruments in combination are powerful tools in the rapid screening and purification of crude samples. The 971-FP software permits direct control of the ELSD via an on-screen user interface (Figure 1), letting the operator change detection settings as and when required. The ELSD response is also used to trigger fraction collection.

Methods and Materials

A solution containing 10 mg/mL of β-carotene, α-tocopherol, δ-tocopherol, 2-aminophenol and 3-aminophenol in hexane was made up and injected onto a SuperFlash™ column.

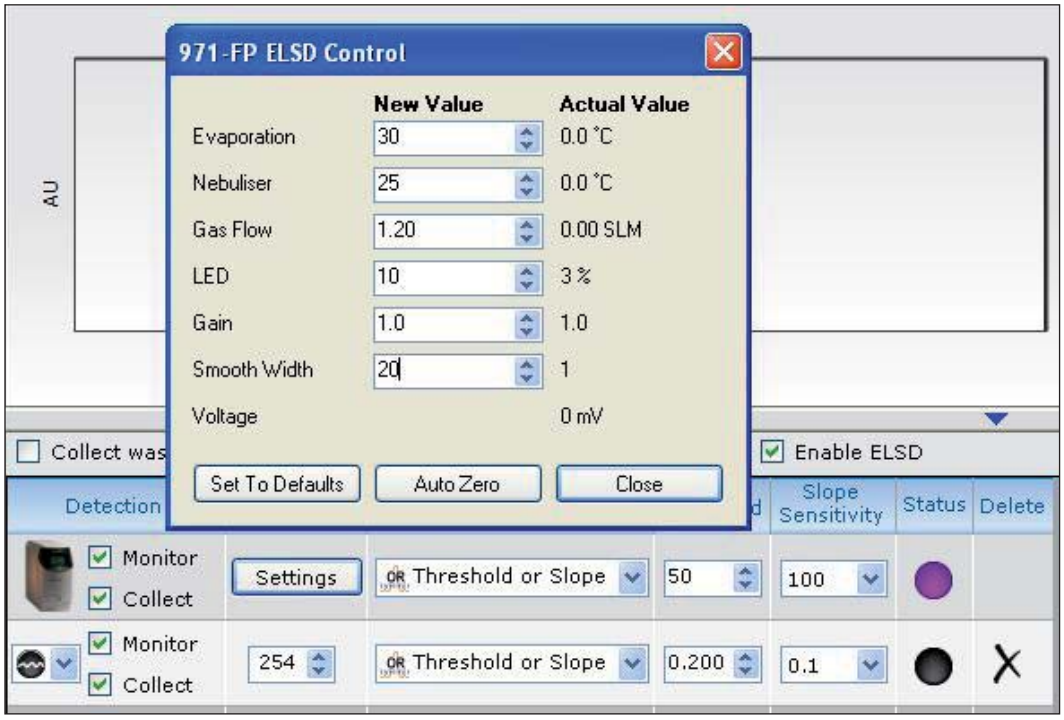

Figure 1. The Varian 971-FP ELSD Control Window.

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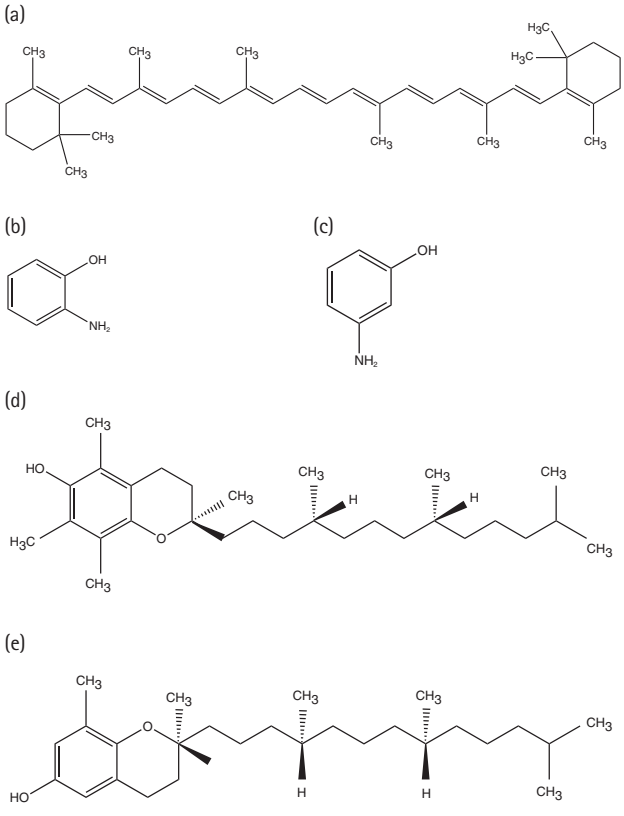

Figure 2. Structures for (a) β-carotene, (b) 2-aminophenol, (c) 3-aminophenol, (d) α-tocopherol, and (e) δ-tocopherol.

Column:	SF15-12g Si 35
Eluent A:	Hexane
Eluent B:	Ethyl acetate
Gradient:	0-100% B in 10 min
Flow Rate:	18.0 mL/min
Injection Volume:	0.5 mL
Temperature:	Ambient
Detection:	UV, 254 nm
	ELSD (neb=15°C, evap=30 °C, gas=1.2 SLM)

Results and Discussion

Figure 3 is an overlay of the different detector responses. The chromatogram was exported directly from the Varian 971-FP and highlighted the importance of using an additional detection technique. The UV response showed only two very small peaks, for 2-aminophenol and 3-aminophenol, and so, if only this trace was recorded, some of the components in the sample would be lost to waste.

The ELSD picked up additional peaks at 1 and 4 minutes, which corresponded to β-carotene, α-tocopherol and δ-tocopherol, respectively. As with the UV traces, fraction collection was also triggered from the ELSD response. Therefore, all components were now isolated. The sensitivity was also significantly increased and the baseline is a lot less noisy as a result.

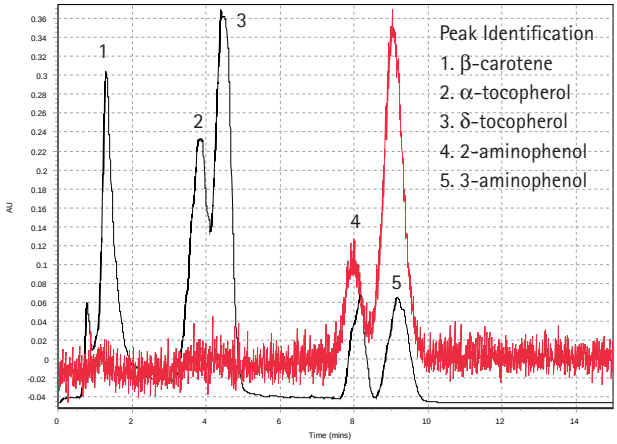

Figure 3. Analysis of UV and non-UV active compounds (UV in red, ELSD in black).

Conclusion

When using flash purification for non-chromophore compounds, the combination of ELSD and UV detectors is a powerful technique that maximizes efficiency. The Varian 971-FP monitors both UV and ELSD responses simultaneously, ensuring that valuable sample is not lost due to poor UV visibility of individual compounds.

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