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# Note

## Laser-light-scattering detection for high-speed counter-current chromatography

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High-speed counter-current chromatography (HSCCC) is a powerful separation technique increasingly used in natural products chemistry [1]. Analytical HSCCC instrumentation has recently become available [2] and applications in natural products chemistry have been reviewed [3]. Several types of detectors have been coupled to analytical HSCCC instruments including UV [4], IR [5] and mass spectrometric (MS) [6] detectors. These systems can provide important structural information about solutes, yet their application to HSCCC is limited by the complexity of instrumentation (MS), high noise levels (UV) or sensitivity (IR).

The use of light-scattering has been suggested as an alternative detection principle in liquid chromatography [7]. Evaporative laser light-scattering detection (ELSD) represents a universal detection method based on the following principle: the eluate [*e.g.* from a high-performance liquid chromatographic (HPLC) column] is nebulized and vaporized, leaving fine particles of solute in a carrier gas stream. These particles pass through a laser beam, scatter the light, and the scattered light is detected. The response is a function of the mass of solute passing through the detector. ELSD has proven particularly useful for carbohydrate and lipid analyses by HPLC [8,9].

In this paper we report the coupling of an ELSD system with analytical HSCCC.

## EXPERIMENTAL

Solvents were HPLC grade (Burdick & Jackson) and water was purified with a Milli-Q system (Millipore). Reference compounds were purchased from C. Roth Inc. (Karlsruhe, F.R.G.). The "micro" high-speed counter-current chromatograph with a 30-ml coil volume and 0.85-mm I.D. PTFE tubing was obtained from P.C. Inc. (Potomac, MD, U.S.A.); solvents were pumped with a Milton Roy laboratory pump;

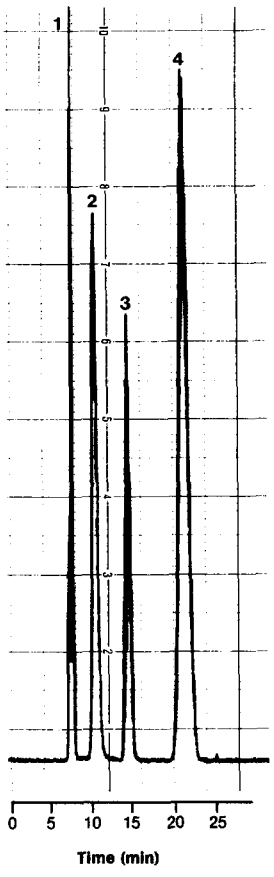
the ELSD system was obtained from Varex (Burtonsville, MD, U.S.A.); chromatograms were recorded on a Varian Model 9176 chart recorder (1 mV full scale).

The HSCCC coil was first filled with stationary phase, then rotation was started while stationary phase was still being pumped. As 1800 rpm was reached, the mobile phase was introduced through the head inlet. When coil equilibrium was reached (effluent changed from displaced stationary phase to mobile phase), the flow-rate was adjusted to 0.8 ml/min. Next, the coil outlet was connected to the light-scattering detector. Complete evaporation of solvent was indicated by a smooth baseline. Compounds **1–4** (see Fig. 1) were dissolved in the stationary phase and the sample solution was injected (time 0 in Fig. 1) by means of a Valco six-port valve and a 0.25-ml sample loop. Quantities injected were 300 μg (**1**), 10 μg (**2**), 35 μg (**3**) and 20 μg (**4**). Back-pressure was below 200 p.s.i., and a stationary phase retention of 90% was determined after the run. The detector was preheated for 20 min; the time constant was 1 s. A back-pressure of 32 p.s.i. nitrogen carrier gas was the result of an arbitrary flow setting of 8 on the detector.

## RESULTS AND DISCUSSION

Fig. 1 shows the separation of a mixture of standard compounds **1–4** [4] by analytical HSCCC. The separation was carried out with the solvent system chloroform–methanol–water (13:7:8) where the lower phase was used as the mobile phase at a flow-rate of 0.8 ml/min. The coil effluent was directly led into the nebulizer compartment of the ELSD system. Nebulizer and exhaust temperatures were set at 110 and 80°C, respectively. This relatively high temperature setting was selected in order to evaporate small amounts of water in the mobile phase and possible droplets of non-retained (aqueous) stationary phase. Carry-over of aqueous stationary phase did not affect the chromatogram. A strong detector response was observed for compounds **2–4** (melting points over 200°C) at 10–35 μg per compound per injection. Compound **1** on the other hand induced a relatively weak detector signal, even at amounts of over 100 μg per injection. A possible explanation for this is that herniarin (**1**) which melts at 117°C, passes through the detector beam as liquid droplets from which incident laser light is not effectively scattered. Signal-to-noise ratios were better than obtained with a photodiode array detector when the same sample mixture was separated [4]. The chromatogram in Fig. 1 shows a smooth baseline, whereas stronger and unexplained detector noise was observed during peak elution.

ELSD is a sensitive and non-specific mass detection method with detection limits in the upper nanogram range [7,8]. However, substances with melting points equal to or smaller than the nebulizer temperature may be difficult to detect, as illustrated in the present application. This has to be taken into consideration if complex samples such as plant extracts are analyzed. Since ELSD is a destructive technique, a flow splitter installed in the eluate line would be needed in order to use this detection method in preparative HSCCC. Detector noise due to carry-over of stationary phase, typical of UV detectors, is eliminated. From our preliminary results it appears that ELSD is an ideal, easy-to-use detection method for analytical HSCCC. Further evaluations using non-UV-absorbing compounds and solvents with high UV cut-off are currently in progress in our laboratory.



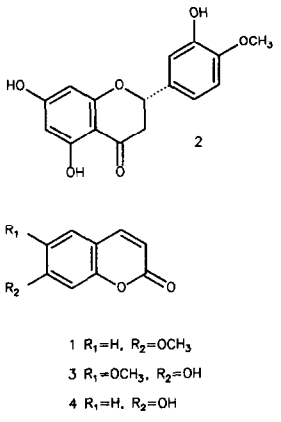




Fig. 1. Analytical HSCCC-ELSD separation of herniarin (**1**), hesperetin (**2**), scopoletin (**3**) and umbelliferone (**4**). Conditions: multilayer coil planet centrifuge (30 ml; 0.85 mm I.D.); solvent, chloroform–methanol–water (13:7:8) with the lower phase as the mobile phase at a flow-rate of 0.8 ml/min.

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