Journal of Chromatography, 558 (1991) 446–450
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 524

Short Communication

Direct coupling of high-speed counter-current chromatography to thin-layer chromatography

Application to the separation of asiaticoside and madecassoside from *Centella asiatica*

B. DIALLO, R. VANHAELEN-FASTRÉ and M. VANHAELEN*

Laboratory of Pharmacognosy and Bromatology, Free University of Brussels, B205-4 Bd. du Triomphe, 1050 Brussels (Belgium)

(First received April 18th, 1991; revised manuscript received May 31st, 1991)

ABSTRACT

The direct coupling of high-speed counter-current chromatography to thin-layer chromatography is proposed as an efficient method for the on-line monitoring of the column effluent. It requires a simple adaptation of the fittings of a Linomat C device without modification of the software. An application to the separation of asiaticoside and madecassoside from a crude extract of *Centella asiatica* is presented.

INTRODUCTION

High-speed counter-current chromatography (HSCCC) has become a widely used method for the isolation of natural products [1]. Commercially available instruments are now very efficient, but simple and effective methods for the on-line monitoring of the column effluent are still lacking. For this purpose, UV, Fourier transform IR, thermospray mass spectrometry and evaporative laser-light scattering detection have been described, but all these methods have disadvantages which limit their application [2,3]; moreover, although in some instances they can provide structural information about the eluted compounds, they do not allow the control of the purity of the eluted fraction. Thin-layer chromatography (TLC) is a very attractive method for this last purpose as the solvent composition used in HSCCC is not a restricting factor and as it allows the combination of many chromatographic adsorbents to specific detection methods, including *in situ* UV spectrometry; however, the manual application of the column effluent on the plates is laborious, especially as the carry-over of the stationary phase, which frequently occurs in HSCCC, leads to

0021-9673/91/$03.50

the decantation of the solvent phases in the collected fractions. The combination of high-performance liquid chromatography (HPLC) with TLC has recently been reported using a sample spray-on apparatus [4]. We report here an adaptation of this device for the coupling of preparative HSCCC to TLC. The method of detection was applied to the separation of asiaticoside and madecassoside, the two main saponins from *Centella asiatica*; purified extracts of this medicinal plant are used to accelerate cicatrizing and grafting.

EXPERIMENTAL

Apparatus

HSCCC was performed using an Ito multi-layer coil separator–extractor [5] (PC. Inc., Potomac, MD, USA) equipped with a 66 m × 2.6 mm I.D. column (column capacity 350 ml). An LDC Milton Roy (Riviera Beach, FL, USA) minipump was used to pump the solvents through the column. The rotational speed was 800 rpm. A manual sample injection valve (Lobar Column Accessories, Merck, Darmstadt, Germany) equipped with a 10-ml loop was used to introduce the sample into the column. A flow splitter was inserted between the restrictor connected at the outlet of the column and the fraction collector. It was made up with a PEEK (polymer compatible with all solvents) three-ports fitting (Alltech, Laarne, Belgium); the original 0.30-mm throughholes of this tee were enlarged to 1.5 mm. Teflon tubing (30 mm × 0.25 mm I.D. × 1.6 mm O.D.) was used for the connection of the splitter to the syringe of a Linomat C device (Camag, Muttenz, Switzerland). Fractions of the eluent were collected using an LKB Ultrorac 7000. The flow diagram of the complete system is presented in Fig. 1.

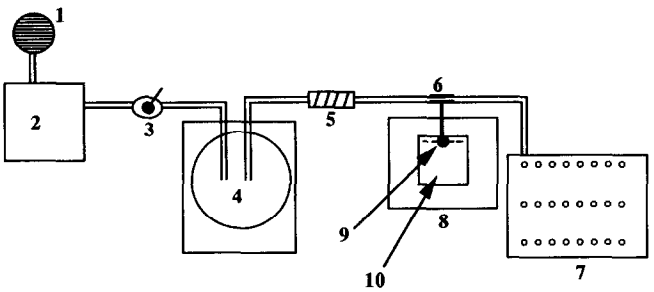

Fig. 1. Top view of the on-line coupling of high-speed counter-current chromatography with thin-layer chromatography. 1 = Mobile phase reservoir; 2 = pump; 3 = injector; 4 = counter-current chromatograph; 5 = restrictor; 6 = flow splitter; 7 = fraction collector; 8 = Linomat C device; 9 = syringe; 10 = chromatoplate.

Separation procedure

A two-phase solvent system was prepared by equilibrating chloroform–methanol–2-butanol–water (7:6:3:4, v/v) at room temperature. After separation, the two phases were degassed in an ultrasonic bath. The upper phase, used as the stationary phase, was pumped into the column at 6 ml/min. After sample injection and the start

of column rotation, the lower phase, used as the mobile phase, was pumped "head to tail" into the column at 4 ml/min.The separation was performed at room temperature and 80 fractions of 12 ml were collected. For the spraying of the effluent onto the silica gel plate, the settings of the Linomat C were as follows: band width, 5 mm; space, 7 mm; rate at which the effluent was aspirated by the syringe, 75 μl/min; peak window, 0.5 min; retention times, 1 min for the first fraction to be collected with an increment of 3 min for each following fraction; the run-time key was pressed when the mobile phase was coming out of the column (fraction 9 ± 1); gas (nitrogen) pressure, 4 bar.

Preparation of sample

Dried mother liquors obtained industrially after crystallization of asiaticoside extracted from the aerial parts of *Centella asiatica* L. were obtained from Syntex (Puteaux, France)). A 400-mg amount of this powder was dissolved in 10 ml of a 1:1 (v/v) mixture of the two phases of the solvent system and the solution was filtered before loading into the column.

Fractionation monitoring

Precoated silica gel $60F_{254}$ plates (10 × 20 cm) (Merck) were developed in an unsaturated tank (22 × 22 × 10 cm) with ethyl acetate–methanol–water (8:2:1, v/v/v) and detection was achieved by spraying a 3% methanolic solution of sulphuric acid and heating at 100°C for 5 min.

RESULTS AND DISCUSSION

The connection of a Camag device recently marketed for the on-line TLC detection in HPLC was modified in order to allow the coupling of HSCCC to TLC. A commercially available three-port fitting was used as a splitter; the through-holes were enlarged to avoid any back-pressure in the HSCCC column. The inside diameter of the tube connecting the splitter to the syringe of the Linomat C device was chosen to allow a suitable aspiration of a homogeneous sample of the effluent by the syringe and to reduce to a minimum the dead volume of the connection. In the proposed application, this dead volume (1.5 μl) represented only 4% of the sprayed volume (37.5 μl) and was negligible. It must be kept in mind that the dead volume could become inconvenient for some applications which require a mobile phase containing large amounts of water and, thus, low sprayed volumes to avoid excessive diffusion of the solution on the adsorbent; this band broadening could also arise when too low concentrations of the sample require high volumes to be sprayed for the subsequent monitoring of the fractionation.

In order to study the potential of the system, the separation of a mixture of triterpenic saponins from an extract of *Centella asiatica* was attempted. A very efficient solvent system was developed on the basis of the partition coefficients of the saponins between the two phases of several solvent systems. Despite the minor structural difference between asiaticoside and madecassoside, the saponins were separated without overlapping of the peaks. The retention of the stationary phase was 63%. Fig. 2 shows the separation obtained with the proposed direct coupling; in order to present a good illustration of the results on the same chromatoplate, the experimental

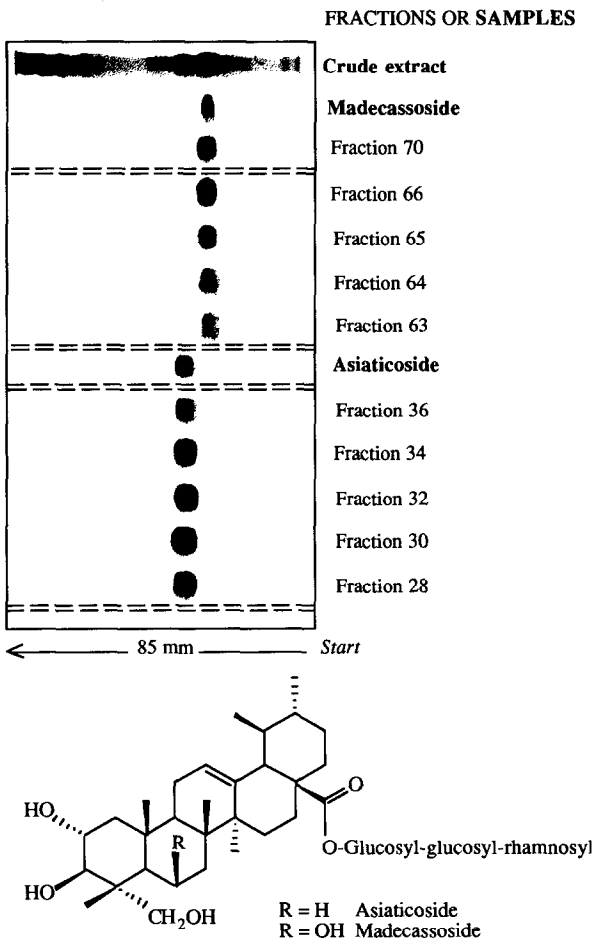

Fig. 2. HSCCC separation of madecassoside and asiaticoside from an extract of *Centella asiatica*. Experimental conditions: column, 66 m × 2.6 mm I.D.; sample size, 400 mg; solvent system, chloroform–methanol–2-butanol–water (7:6:3:4, v/v); mobile phase, lower non-aqueous phase; flow-rate, 4 ml/min; rotation speed, 800 rpm; detection, on-line effluent spraying on silica gel plate followed by TLC [solvent, ethyl acetate–methanol–water (8:2:1, v/v/v); detection, 3% methanolic solution of sulphuric acid and heating at 110°C for 5 min].

conditions related to the effluent spraying were modified in order to insert the bands corresponding to the standards and the starting sample and to reduce the track number. In usual experimental conditions, the apparatus software allows the running of the same effluent spraying program for several other chromatoplates.

The combination of the present method of detection with other detection systems such as on-line UV absorptiometry could improve the automation of the system. However, our results indicate that simple on-line TLC detection in HSCCC is a powerful and inexpensive method which avoid the tedious manual spotting of the numerous eluted fractions and which affords useful information about both peak purity and separation achievement.

ACKNOWLEDGEMENTS

We thank Mr. R. Maillard (Laboratoires Syntex, Puteaux, France) for the generous gift of asiaticoside and of a crude mixture of madecassoside and asiaticoside. B. Diallo thanks the Administration Générale de la Coopération au Développement (AGCD) for financial support.

REFERENCES

1 K. Hostettman, M. Hostettmann and A. Marston, *Preparative Chromatographic Techniques*, Springer, New York, 1986, pp. 80–126.
2 D. E. Schaufelberger, T. G. McCloud and J. A. Beutler, *J. Chromatogr.*, 538 (1991) 87; and references cited therein.
3 S. Drogue, M.-C. Rolet, D. Thiebaut and R. Rosset, *J. Chromatogr.*, 538 (1991) 91; and references cited therein.
4 D. E. Jaercchen, in H. Traitler, A. Studer and R. E. Kaiser (Editors), *Proceedings of the 4th International Symposium on HPTLC, Planar Chromatography*, Selvino, Rad Dünkheim, 1987, p. 815.
5 V. Ito, *Anal. Chem.*, 17 (1986) 65.