Two dimensional chromatography: LC-GC online coupling of an Agilent 1260 Infinity LC and an Agilent 7890A GC

Technical Overview

Authors

Rebecca Esche, Andreas Barnsteiner, Karl-Heinz Engel
Technische Universität München
Lehrstuhl für Allgemeine Lebensmittelteltechnologie, Maximus-von-Imhof-Forum 2, 85350 Freising, Germany

Walter Kohlert, Stefan Fenzel
Agilent Technologies Inc.,
Waldbronn, Germany

Abstract

This Technical Overview describes the online coupling of an Agilent 1260 Infinity LC and an Agilent 7890A GC via an Agilent 1200 Infinity Series 2-position/6-port switching valve using the solvent vent mode of the multimode inlet of the GC for solvent evaporation. The GC chromatogram of a cholesteryl fatty acid ester mixture obtained by the online LC-GC combination was comparable to that obtained using the Agilent 7890A GC as a single GC with split injection.

Introduction

On-line coupled LC-GC has been applied successfully to the analysis of complex samples, e.g. the investigation of steryl esters in fats and oils[1-3]. Coupling LC with GC allows the combination of the best features of both techniques: the LC part can be used for selective clean-up, fractionation and pre-concentration of samples. The subsequent GC analysis of the LC fraction of interest offers high separation efficiency and a variety of selective detection methods. The sample preparation and the analysis take place in a closed, usually automated system. The risks of sample loss and contamination are minimized. The combination allows a fast analysis (costs were reduced) with high reliability and repeatability[4-6].

Agilent Technologies

Coupling the two technologies is not trivial, as the two separation techniques operate in phases that are in two different physical states. The introduction of a large amount of solvent into a GC system requires the use of special techniques/interfaces to eliminate the large solvent volumes (up to several hundred µL), and to fix the soluted analytes at the entrance of the GC separation column as a sharp band[4-6]. Techniques such as (i) on-column, (ii) loop-type, and (iii) vaporizer interfaces are used. Using on-column and loop-type interfaces, the solvent evaporation is performed in the GC capillary column through a pre-column system in combination with an early solvent vapor exit. Vaporizer interfaces, like hot vaporizing chambers or programmable temperature vaporization (PTV), allow the elimination of the solvent prior to the GC capillaries; in this case, a solvent vapor exit is not necessary.

The Agilent Multimode Inlet (MMI) of the Agilent 7890A GC offers options for the introduction of large sample volumes, up to more than 250 µL. A standard Agilent 7890A GC can be used for the coupling of LC and GC.

Instrumental

An Agilent 1260 Infinity LC was used. Interfacing was done with an Agilent 1200 Series 2-position/6-port switching valve which was equipped with a 200 µL sample loop (Figure 1). The valve was controlled by the LC software. In addition to the regular MMI-gas supply line, a second line was installed to enable a pressure-controlled transfer. The latter was controlled by the PCM-C module. The GC run was started by a contact closure 0.05 minutes before the transfer of the LC fraction was performed by switching the Agilent 1200 Series 2-position/6-port switching valve. The evaporation of the eluent was performed using the temperature programmable MM Inlet in the PTV solvent vent mode.

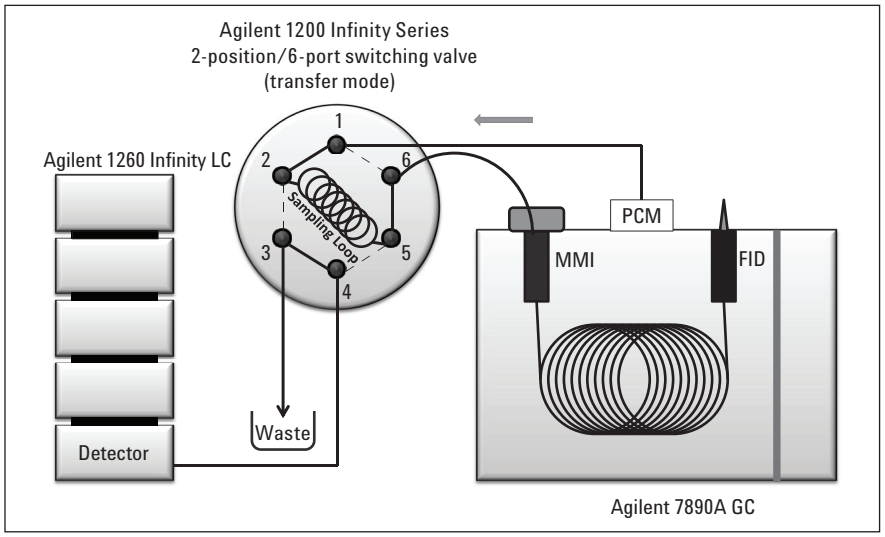

Figure 1
On-line coupling of the Agilent 1260 Infinity LC to the Agilent 7890A GC via an Agilent 1200 Series 2-position/6-port switching valve.

Chromatographic conditions

LC conditions		
Injection volume:	2 µL	
Eluent:	*n*-hexane/*tert*-butylmethyl ether (96:4, v/v)	
Column flow:	0.200 mL/min	
Column type:	Eurospher-100Si (250 mm × 2 mm id, 5 µm)	
Wavelength:	205 nm	
LC controlled interface		
Transfer valve:	4.25 min:	Position 1 → 2
	7.50 min:	Position 2 → 1
GC start:	4.20 min:	Change contacts switch contact A to closed
	4.25 min:	Change contacts switch contact A to open
GC conditions		
Front MM inlet:	Mode:	Solvent vent
	Carrier:	H_2
	Pressure:	7.8 psi
	Septum purge flow:	3 mL/min
	Vent pressure:	4 psi until 0.5 min
	Vent flow:	1000 mL/min
	Temperature program:	Initial: 50 °C for 0.5 min
		Rate 1: 900 °C/min to 350 °C for 2 min
	Purge flow to split vent:	2.5 mL/min at 0.5 min
	Gas saver:	20 mL/min after 5 min
Column number 1:	Column type:	Restek Rtx-200MS: 30 m × 250 µm; 0.1 µm d_f;
	Constant flow:	1.5 mL/min
Column number 2:	Transfer line, controlled by PCM C-1	
	Pressure program:	Initial: 5 psi for 0.3 min
		Rate 1: 10 psi/min to 20 psi
Oven:	Temperature program:	Initial: 40 °C for 2 min
		Rate 1: 100 °C/min to 100 °C for 0 min
		Rate 2: 15 °C/min to 310 °C for 2 min
		Rate 3: 1.5 °C/min to 340 °C for 3 min
Detector:	FID: 360 °C	
	(H_2: 30 mL/min, Air: 400 mL/min; Makeup: 25 mL/min)	

Table 1
LC and GC conditions.

Results and discussion

The established parameters (Table 1) allowed the coupling of an Agilent 1260 Infinity LC and an Agilent 7890A GC by means of the multimode inlet, which allows the introduction of the sample, for example, in an SSL- or in a solvent vent mode. The latter was used for the transfer of the large LC fraction. The applicability of the introduced instrumentation was exemplarily demonstrated for a mixture of cholesteryl fatty acid esters (C12, C16, C18, C20, and C22). The chromatograms are shown in Figure 2. The GC chromatogram of the cholesteryl ester mixture analysis by means of the single GC (2 µL I.V.; inlet temperature: 280 °C; split ratio 1:15) was similar to that obtained after the transfer of the LC-fraction by means of the on-line coupled system.

The LC-GC transfer can be performed by connecting the carrier gas supply line directly via the transfer valve into the MMI or installing a second line, which can be controlled by the PCM-C module as shown in Figure 1. The latter allows the setting of a ramp to control the pressure in the transfer line during the transfer and also during the MMI-program. The transfer/injection speed into the MMI can be controlled and pushing back of solvent vapors into the transfer line can be avoided. The insertion depth of the stainless steel transfer line into the inlet was as low as possible to minimize the metal surface in the MMI.

The Solvent Elimination Calculator of the Agilent ChemStation software was a helpful tool to determine the required starting conditions for the best solvent evaporation. During the transfer (solvent vent mode) the inlet temperature (50 °C) was below the solvent boiling point. To vaporize the 200 µL organic eluent, the vent flow was set to 1,000 mL/min with a pressure of 4 psi until 0.5 min. At 0.5 min the inlet was switched to the purge mode with a purge flow of 2.5 mL/min to split vent. The inlet was then heated up fast (900 °C/min to 350 °C) to enable an effective transfer of the analytes onto the GC column. Minor changes of these settings led to significant changes in the sensitivity and degradation of the analytes. Increasing the vent or purge flow resulted in a decreased GC response. Due to lower vent or purge flows, the evaporation of the solvent was insufficient. Higher solvent amounts were transferred to the column and resulted also in lower sensitivity.

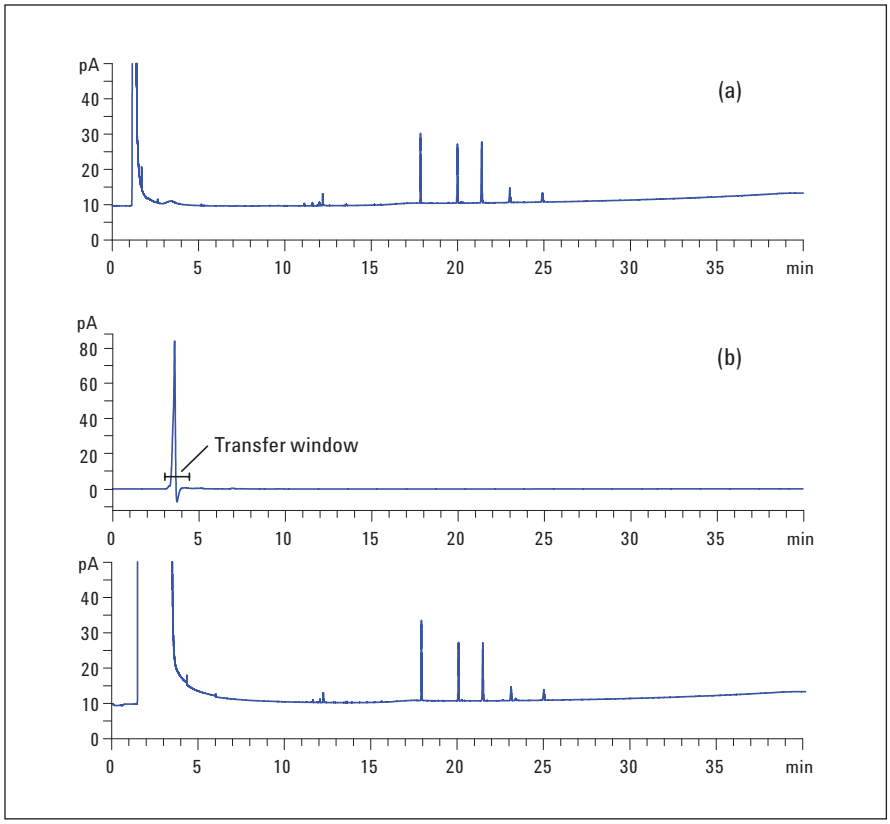

Figure 2
Analysis of a cholesteryl ester mixture by means of (a) a single GC and (b) an on-line coupled LC-GC (the upper chart shows the LC- and lower chart the GC-chromatogram of the transferred LC-fraction)

The Agilent 5183-4647 was the most qualified liner for the determination of cholesteryl fatty acid esters. Other liners tested (Agilent 5190-2296; Agilent 5062-3587; Agilent 5183-4711) resulted in lower reproducibility and sensitivity.

Conclusion

On-line coupling of an Agilent 1260 Infinity LC and an Agilent 7890A GC was shown to be suitable for the analysis of cholesteryl fatty acid esters. This combination may be applied to the determination of plant steryl esters in complex matrices like vegetable oils or in functional foods, for example, enriched margarines.

References

1.
K. Grob, M. Lanfranchi, C. Mariani, "Evaluation of olive oils through the fatty alcohols, the sterols and their esters by coupled LC-GC." *J. Am. Oil Chem. Soc.* **1990**, 67, (10), 626-34.

2.
W. Kamm, F. Dionisi, L. B. Fay, C. Hischenhuber, H. G. Schmarr, K. H. Engel, "Analysis of steryl esters in cocoa butter by on-line liquid chromatography-gas chromatography," *J. Chromatogr A,* **2001**, 918, (2), 341-349.

3.
A. Miller, T. Frenzel, H. G. Schmarr, K. H. Engel, "Coupled liquid chromatography-gas chromatography for the rapid analysis of γ-oryzanol in rice lipids," *J. Chromatogr A,* **2003**, 985, (1-2), 403-410.

4.
P. Dugo, G. Dugo, L. Mondello, "On-line coupled LC-GC: theory and applications," *LC-GC Eur.* **2003**, 16, (12a), 35-43.

5.
T. Hyoetylaeinen, M. L. Riekkola, "On-line coupled liquid chromatography-gas chromatography," *J Chromatogr A,* **2003**, 1000, (1-2), 357-84.

6.
T. Hyoetylaeinen, M. L. Riekkola, "Perspectives of on-line coupled liquid chromatography-gas chromatography," *Anal. Bioanal. Chem.* **2004**, 378, (8), 1936-1938.

www.agilent.com/chem/lc

Published in USA, August 1, 2011
Publication Number 5990-8025EN

Agilent Technologies