Developing a green LC method for the determination of the furocoumarins 5-MOP and 8-MOP in citrus oils using the Agilent 1290 Infinity LC

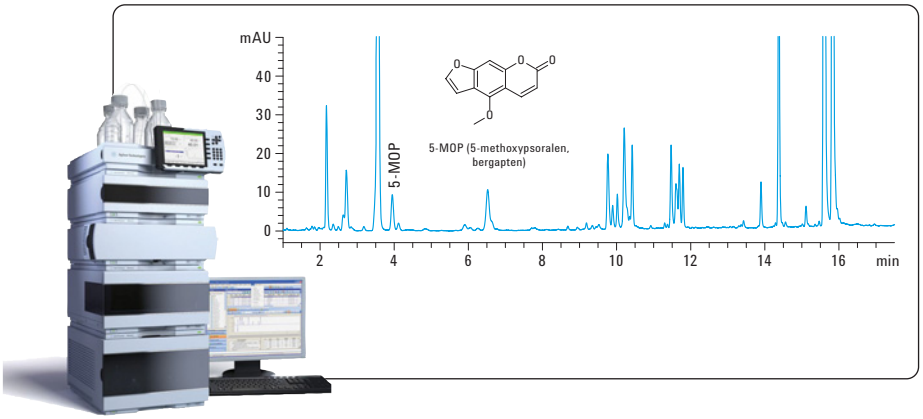
Application Note

Natural Products, Fragrances

Authors

Gerd Vanhoenacker, Frank David, and Pat Sandra
Research Institute for Chromatography
Kennedypark 26
B-8500 Kortrijk
Belgium

Bernd Glatz and Edgar Naegele
Agilent Technologies R & D and Marketing GmbH and Co. KG
Hewlett-Packard-Str. 8
76337 Waldbronn
Germany



Abstract

Lemon and orange oils were analyzed for the presence of the furocoumarins 5-methoxypsoralen (5-MOP) and 8-methoxypsoralen (8-MOP) with the Agilent 1290 Infinity LC system. At 1000 bar, the total analysis time in reversed-phase (RP) liquid chromatography (LC) with acetonitrile and water as mobile phase components could be reduced to 4 min. In the frame work of green chromatography, and the present acetonitrile shortage, mobile phases constituted of water/methanol and water/ethanol were compared to water/acetonitrile. The influence of the nature of organic modifiers on selectivity and peak capacity was investigated. The figures of merit of the different methods were compared for the determination of 5-MOP in a lemon oil sample containing approximately 50 mg/kg. 8-MOP was not detected at the ppm level. The detection limit for both furocoumarins was approximately 1 mg/kg citrus oil.

Agilent Technologies

Introduction

Furocoumarins are natural products that may be present in plant extracts and essential oils used in fragranced cosmetic products. The furocoumarins have been identified as photomutagenic and photocarcinogenic products. The International Agency for Research on Cancer (IARC) has classified 5-MOP (5-methoxypsoralen, bergapten) and 8-MOP (8-methoxypsoralen, xanthotoxin) when combined with UV radiation as group 2A (probably carcinogenic to humans) and as group 1 (carcinogenic to humans) risk carcinogens, respectively.

On that basis, limits have been defined for the presence of psoralens in cosmetics. The Commission Directive 95/34/DC of 1995 states that furocoumarins should be below 1 mg/kg (1 ppm) in sun protection and in bronzing products.[1] There is an ongoing debate to extend this 1 ppm limit to all finished cosmetic products.[2] Therefore, fast analysis of furocoumarins in cosmetics and in the essential oils used in cosmetics is of utmost importance.

The Agilent 1290 Infinity LC system in combination with UV detection was evaluated for the determination of 5-MOP and 8-MOP (Figure 1) in a lemon and orange oil sample. Typical analysis times for such samples using standard LC instrumentation are 30 min.[3] The possibility of increasing the analysis speed by employing the high pressure capabilities of the Agilent 1290 Infinity LC was investigated. Mobile phases for these analyses are commonly composed of water and acetonitrile. Different organic modifiers (acetonitrile, methanol, and ethanol) were compared and a green method with biodegradable ethanol as the mobile phase constituent was developed.

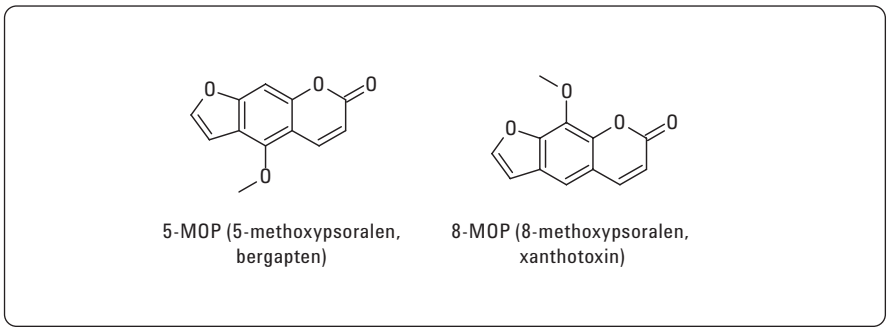


Figure 1
Structures of 5-MOP and 8-MOP.

The features of diode array detection (DAD) and mass spectrometer (MS) detection for trace analysis of furocoumarins using the Agilent 1290 Infinity LC will be described elsewhere.[4]

Experimental

Instrumentation and method

An Agilent 1290 Infinity LC system with the following configuration was used:

Part number	Description
G4220A	Agilent 1290 Infinity Binary Pump with integrated vacuum degasser
G4226A	Agilent 1290 Infinity Autosampler
G1316C	Agilent 1290 Infinity Thermostatted Column Compartment
G4212A	Agilent 1290 Infinity Diode Array Detector

Method parameters:	
Column	C18 150 mm L × 2.1 mm id, 1.7 µm d_p C18 100 mm L × 2.1 mm id, 1.7 µm d_p
Mobile phase	A = Water B = Acetonitrile, methanol or ethanol
Flow rate	Variable
Gradient	Variable
Temperature	80 °C
Injection	1 µL
Detection	DAD, Signal 315/4 nm, Reference 500/60 nm, 40 Hz

Solutions and samples

Stock solutions of 500 µg/mL of 5-MOP and 8-MOP standards were prepared in ethanol. The solutions were further diluted in ethanol prior to injection. Samples of lemon and orange oil were used. The oils were analyzed separately, as well as mixed, and they were diluted 1/10, volume to volume, in ethanol prior to injection.

Results and Discussion

Increase speed

The high pressure capabilities of the Agilent 1290 Infinity LC system were utilized to increase speed of analysis. Starting from a RP-LC method with a water/acetonitrile gradient, the flow rate and gradient slope were increased proportionally to each other while the isocratic hold time at the beginning of the analysis was reduced to maintain the elution profile. The flow rate was increased from 0.45 to 0.6, 0.9, and 1.2 mL/min resulting in pressure drops up to 1090 bar on the 150 mm long column. The analysis time was more than 2.5 times faster and could be obtained without sacrificing resolution.
Figures 2A, B, and C show some typical profiles for the mixed oil sample. Only 5-MOP was detected in the sample. Only use the analysis conditions in Figure 2C if your column and hardware is rated above 1000 bar.

A further increase in speed could be obtained by reducing the column length to 100 mm and increasing the flow rate to 1.45 mL/min (Figure 2D). The result is that the analysis time is approximately 4 min with a pressure of 1000 bar. The drawback of using a shorter column for high speed analysis is that the resolution decreases as well. However, the separation is still sufficient to detect 5-MOP in the sample.

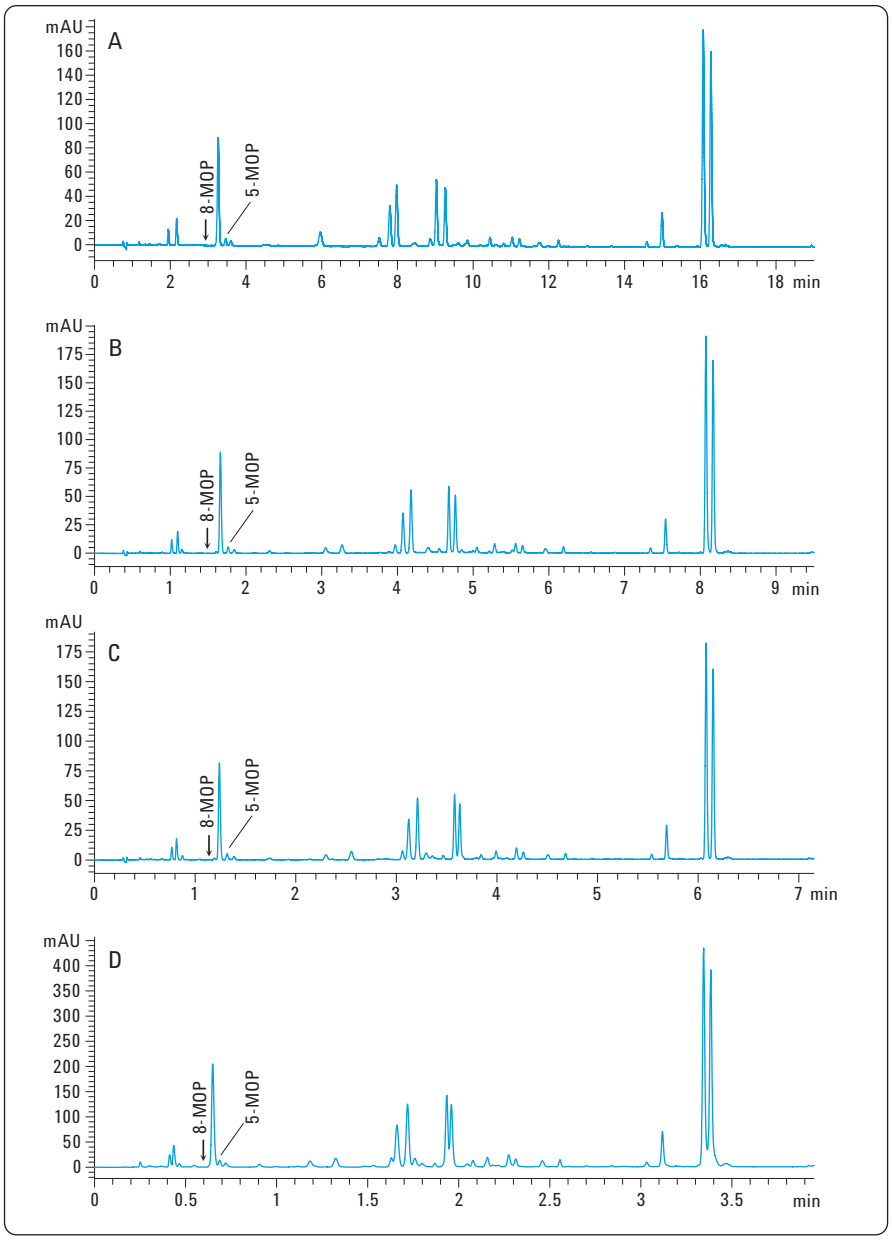


Figure 2
Chromatograms for the analysis of the mixed oil sample with different flow rates and columns. Flow rate/column length: 0.45 mL/min/150 mm (A), 0.9 mL/min/150 mm (B), 1.2 mL/min/150 mm (C), and 1.45 mL/min/100 mm (D). Mobile phase: water-acetonitrile.

Conditions Figure 2	A	B	C	D
Column length	**150 mm**	**150 mm**	**150 mm**	**100 mm**
Flow rate	**0.45 mL/min**	**0.9 mL/min**	**1.2 mL/min**	**1.45 mL/min**
Gradient	0–5 min: 30% B isocratic 5–25 min: 30–100% B	0–2.5 min: 30% B isocratic 2.5–12.5 min: 30–100% B	0–1.9 min: 30% B isocratic 1.9–9.4 min: 30–100% B	0–1 min: 30% B isocratic 1–5.2 min: 30–100% B
Maximum pressure	**440 bar**	**840 bar**	**1090 bar**	**1000 bar**

Green chromatography

In a second application, the Agilent 1290 Infinity LC system was used to develop a green LC method. The citrus oil was analyzed with three different organic modifiers. Solvent composition and gradients had to be adapted for each combination to obtain a similar elution window. The results are shown in Figure 3. The elution profile showed significant differences with the sample analyzed in section 1 (mixed oil sample). The reason is that the orange oil contains mainly polymethoxylated flavanoids while the lemon oil is mostly composed of psoralene derivatives.

Analyzing samples with only water and ethanol as mobile phase components is green chromatography. This approach is interesting ecologically as well as economically due to the present acetonitrile shortage. The drawback of using water/ethanol mobile phases is the high backpressure that is generated. For this particular analysis the pressure reached 770 bar with ethanol while only 440 and 590 bar for acetonitrile and methanol, respectively. The Agilent 1290 Infinity LC is rated to 1200 bar and has no problem with these high backpressures.

The presence of 5-MOP could be elucidated in the 3 chromatograms. This indicates that in the mixed sample, 5-MOP was originating from the lemon oil sample and not from the orange oil sample. Note that, identification and quantification of all furocoumarins at trace levels is best performed by using mass spectrometry.[4]

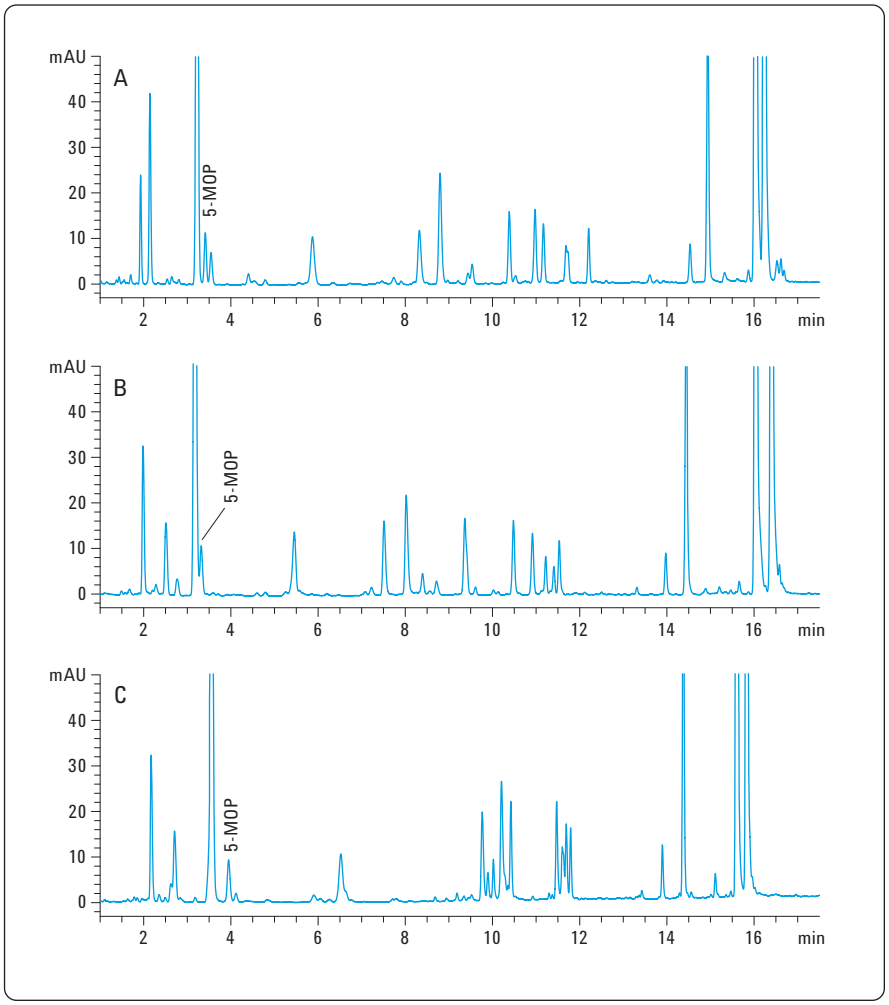


Figure 3
Chromatograms for the analysis of a lemon oil sample with 3 different modifiers. Acetonitrile (A), methanol (B), and ethanol (C). Flow rate: 0.45 mL/min.

Conditions Figure 3	A	B	C
Modifier	**Acetonitrile**	**Methanol**	**Ethanol**
Gradient	0-5 min: 30% B isocratic 5–25 min: 30-100% B	0–3.5 min: 40% B isocratic 3.5–23.5 min: 40-100% B	0–5 min: 23% B isocratic 5–25 min: 23–100% B
Maximum pressure	**440 bar**	**590 bar**	**770 bar**

Quantitative and performance data

The different methods were applied to perform the quantitative analysis of 5-MOP in the lemon oil sample and their performances were compared (Table 1). Calibration lines were created by single consecutive injections of standard solutions containing 0.1, 0.5, 1, 5, and 10 µg/mL of 5-MOP. The data are summarized in Table 1.

In order to compare the resolving power of the different methods the peak capacity was calculated for each. This was done by dividing the gradient time with the average peak width at the base (4σ) determined for the 5 µg/mL 5-MOP solution. As expected, the resolving power on the shorter column decreased compared to the 150-mm column. It is noteworthy that the peak capacity with the water/acetonitrile was not affected by the increased flow rate while peak capacities for acetonitrile and ethanol were similar.

The assay of 5-MOP was very similar with all methods and the relative standard deviation (RSD) was 1.98%. In the sample solution an average concentration of 5.46 µg/mL was detected. This corresponds to 51 mg/kg 5-MOP in the original lemon oil.

Mobile phase	Column length (mm)	Flow rate (mL/min)	Maximum pressure (bar)	Peak capacity	Linearity (R^2)	Assay 5-MOP (µg/mL)*
Acetonitrile	150	0.45	440	219	>0.9999	5.39
Methanol	150	0.45	590	192	>0.9999	5.60
Ethanol	150	0.45	770	215	>0.9999	5.30
Acetonitrile	150	0.90	850	218	>0.9999	5.53
Acetonitrile	150	1.20	1090	213	>0.9999	5.44
Acetonitrile	100	1.45	1000	152	>0.9999	5.52
					Average	**5.46**
					RSD (%)	**1.98**

*Concentration in sample solution. The oil is diluted 1/10 v/v in ethanol prior to analysis.

Table 1.
Comparison of the different methods. Assay was performed on the lemon oil sample.

Conclusion

The Agilent 1290 Infinity LC system was used to analyze furocoumarins in citrus oil samples. The performance of the original method with a water/acetonitrile mobile phase was compared to green chromatography methods where the acetonitrile was replaced with methanol or with ethanol. Additionally, the performance of a high speed method was evaluated. This Application Note demonstrates that the high pressure capabilities and the high detector acquisition rate of the Agilent 1290 Infinity LC system are very useful tools for increasing analysis speed. In addition, it shows that the Agilent 1290 Infinity LC system can be used for methods that are less toxic and more environmentally friendly than existing methods.

References

1.
Eightieth Commission Directive 95/34/EC of 10 July 1995 adapting to technical progress Annexes II, III, VI and VII to Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products (1995)

2.
Sixth plenary of 13 December 2005, Scientific Committee on Consumer Products SCCP/0942/05, Opinion on furocoumarins in cosmetic products (2005)

3
Frérot E., Decorzant E., *J. Agric. Food Chem*. 52 (2004) 6879-6886.

4.
G. Vanhoenacker, F. David, P. Sandra, in preparation.

www.agilent.com/chem/1290

May 1, 2009
Publication Number 5990-4033EN

Agilent Technologies