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Short communication

Separation of polymer and on-line determination of several antioxidants and UV stabilizers by coupling size-exclusion and normal-phase high-performance liquid chromatography columns

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Abstract

A procedure consisting of connecting in series two different HPLC columns, one for size-exclusion chromatography (SEC) and the second one a normal-phase (silica) column has been developed. An automatic three-way switching valve was placed between the two columns. Through the valve, the polymer was drained whereas the rest of the compounds, a group of antioxidants and UV stabilizers, were separated and analyzed in the second column. The behaviour of the SEC column in different organic phases is studied. Detection limits about 0.1 $\mu g\ ml^{-1}$ were obtained for BHT, Tinuvin 326 and Tinuvin 327; 0.2 $\mu g\ ml^{-1}$ for Irganox 1076, and 1.1 $\mu g\ ml^{-1}$ for Cyasorb UV 9 and Cyasorb UV 1084. R.S.D. values of the whole process are lower than 4%.

1. Introduction

The determination of additives such as antioxidants and UV stabilizers in plastic packaging materials in contact with food is carried out by means of an extraction step, in which the compounds contained in the polymer are transferred to the organic solvent. The extraction can be carried out by classical extraction, in which the organic solvent is shaken with the polymer, by Soxhlet (continuous extraction) or by an ultrasonic bath. The ultrasonic bath has been shown as one of the most efficient systems in which most of the components present in the packaging material are transferred to the liquid organic phase. As a result of this extraction step, all the additives and small molecules, some oligomers and sometimes the polymer, can be present in the organic solution obtained. Obviously, a second step of clean-up is necessary to achieve the separation of the compounds of interest. Several methods have been proposed for the clean-up of such organic solutions [1–3] but among them, size-exclusion chromatography (SEC) is the most frequently used [4–8] due to its simplicity and short analysis times.

In most cases, the interfering compounds have larger sizes than the additives and consequently, they are eluted from the SEC column before the additives. However, SEC columns elute the compounds grouped in bands, instead of in narrow peaks corresponding to individual com-

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pounds. Consequently, a second separation column is necessary.

Fraction collectors connected to SEC columns are a useful alternative to facilitate the second separation step. Once the desired fraction is concentrated, it can be analyzed either by gas chromatography, reversed-phase [9–16] or normal-phase [14–20] high-performance liquid chromatography, but in all cases this behaviour implies working in batch, which is complex and time consuming.

The possibility of connecting on-line two HPLC columns with different stationary phases has been tried by different authors [21–28]. Nevertheless, this is not an easy task. First, the mobile phase has to be compatible with both columns, and secondly, the major compounds separated in the first column, e.g. SEC, should not be introduced into the second column. Johnson et al. [29] described a coupled column chromatography connecting size-exclusion and a reversed-phase columns each one with a different mobile phase for the analysis of some additives in rubber. Two independent HPLC systems were necessary to achieve the effective coupling, in which the first pump worked with tetrahydrofuran (THF) in the isocratic mode, with the SEC column and a UV variable-wavelength detector; the second system pumped water–acetonitrile through a gradient with a reversed-phase column and another UV detector. Between the columns, a switching valve acts as injector of the second HPLC system. This is a very powerful approach although two HPLC systems and a switching valve are necessary.

The present paper shows another system which allows the separation of polymer and the analysis of antioxidants and UV stabilizers in organic media in only one step. The system consists of two HPLC columns in series, one for SEC and the other one a normal-phase (silica). Between them, an automatic switching valve permits the polymer being drained at controlled time. Once the major compounds are eliminated by one of the valve ways, after the switch the rest of compounds passes through the other way to the second column, where their separation is improved and they can be quantified properly. The behaviour of each column and the analytical features for the determination of several antioxidants and UV stabilizers in polymers are discussed.

2. Experimental

2.1. Reagents

2,6-Di-*tert.*-butyl-4-metylphenol (BHT) was from Fluka (Buchs, Switzerland), pure quality, 2-hydroxy-4-methoxybenzophenone (Cyasorb UV 9), styrene and benzophenone were from Sigma (St. Louis, MO, USA), analytical-reagent quality; octadecyl-3,5-di-*tert.*-butyl-4-hydroxyhydrocinnamate (Irganox 1076), 2,2′-thiobis(4-*tert.*-octylphenolate)-*n*-butylamine nickel (Cyasorb UV 1084), 2(3′-*tert.*-butyl-2′-hydroxy-5′-methylphenyl)-2H-5-chlorobenzotriazole (Tinuvin 326) and 2-(2′-hydroxy-3′,5′-di-*tert.*-butylphenyl)-2H-5-chlorobenzotriazole (Tinuvin 327), were supplied by courtesy of Ciba-Geigy (Basle, Switzerland). All of them were used without further purification. Polystyrene was from Aldrich (Steinheim, Germany), average M_r 280 000. Chloroform, cyclohexane, dichloromethane, *n*-hexane and tetrahydrofuran (without stabilizer) were from Merck (Darmstadt, Germany), HPLC quality. Alugram Nano-SIL G/UV_{254} thin-layer chromatography (TLC) plates were from Macherey–Nagel (Düren, Germany).

2.2. Apparatus

A Kontron Instruments liquid chromatograph (Milan, Italy) with two pumps 420, autosampler 460, oven controller 480, dual UV–Vis detector 430 and 80286 personal computer with Data System 450, version 1.85 was used.

2.3. Procedures

Two on-line coupled columns were used: a Hewlett-Packard (Palo Alto, CA, USA) PL-Gel 50 Å, 300 × 7.5 mm I.D. with pre-column (same characteristics but 50 × 7 mm I.D.), and a Scharlau (Barcelona, Spain) Nucleosil 100-7 OH, 7

μm, 25 × 4.6 mm I.D. Two UV wavelengths were set, 280 and 254 nm. The mobile phase composition was *n*-hexane–dichloromethane (73:27, v/v). The flow-rate was 0.9 ml min^{-1}. The oven temperature was 35°C in all cases.

To allow the separation of residual polymer from the studied compounds, a Rheodyne (Cotati, CA, USA) Model 7030 ARV three-way valve with electric two-position actuator (Thar Designs, Pittsburgh, PA, USA) was used. The automatic control of this valve was via software.

Solvents were degassed by ultrasonic bath (15 min) and they were filtered through PTFE 0.45-μm filters before use. A 10-μl volume of the THF sample solution was injected into the column. Broadening appeared in peak shapes when injecting larger volumes (more than 20 μl), due to the higher polarity of THF compared with the mobile phase used. Alternatively, standard solutions of the compounds dissolved in mobile phase were injected without problems.

Samples were also filtered through syringe PTFE filters (luer lock type, 0.45 μm) before its injection in the HPLC system.

Spiked samples of polymer and antioxidants were prepared as follows: 0.01 g of polystyrene were added to 10 ml dichloromethane solution which contained 25 μg ml^{-1} of each antioxidant and UV stabilizer. This solution was used to optimize the analytical procedure with the two columns connected in series.

3. Results and discussion

3.1. Behaviour of the SEC column

The stationary phase used in SEC is usually a copolymer of styrene–divinylbenzene which has a neutral behaviour against mobile phases as THF, dichloromethane, chloroform or N,N-dimethylformamide, although these gels can be used in a wide range of polarities from cyclohexane to acetonitrile or methanol. The common mechanism of separation is size-exclusion, and the solvent used as mobile phase has the ability of swelling up the stationary phase. Consequently, the pore size changes and the separation capacity is modified, too. This effect is shown as a variation of the retention time of each compound.

However, when using lower-polarity solvents such as *n*-hexane, cyclohexane or mixtures of them with those previously cited, an adsorption effect appears as was described before in different works with SEC [30–32]. This has been confirmed by studying the behaviour of benzophenone and styrene. So, the plot of retention times differences (directly related with the resolution) vs. the percentage of *n*-hexane in the mixture used as mobile phase showed that when the percentage of *n*-hexane is lower than 70%, the predominant mechanism is size-exclusion, whereas at higher proportions of *n*-hexane the adsorption effect is very clear.

3.2. Determination of antioxidants and UV stabilizers

Once the behaviour of the SEC column was established, the determination of some antioxidants and UV stabilizers commonly used in packaging materials for food contact was carried out. All the compounds studied have a similar chemical structure. In consequence, the SEC column is not enough to achieve their analytical separation. When two identical SEC columns were connected in series, the resolution was slightly improved, but still insufficient. Furthermore, under these conditions, the time of analysis was doubled in comparison to that when using only one column.

As one of the major components in the packaging materials is the polymer, the analytical procedure involves the separation of the polymer from the rest of the studied compounds, and this first separation was successfully achieved by the SEC column.

In order to get the whole process, separation of major components, usually called clean-up, and analytical separation in only one step, two different HPLC columns were connected in series. The first one was the SEC column, and the second one a normal-phase (silica) column. A three-way switching valve was connected between the two columns, as shows Fig. 1. Both

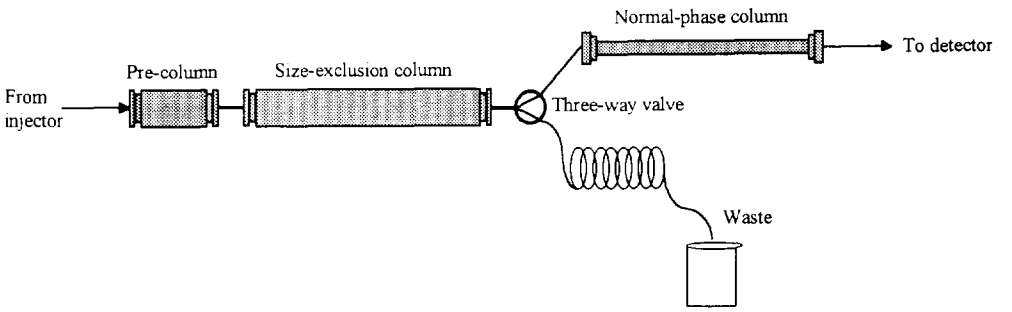


Fig. 1. Diagram of the system with SEC and normal-phase columns and a switching valve between them for on-line clean-up and analysis of antioxidants and UV stabilizers from polymer extracts.

columns are compatible with the organic mixture of *n*-hexane and dichloromethane used as mobile phase. The ratio of *n*-hexane and dichloromethane was previously optimized by TLC using UV light to show the obtained spots. An optimum ratio of dichloromethane–*n*-hexane (27:73) was found.

Fig. 2a shows the chromatogram obtained when a solution containing polystyrene and a mixture of antioxidants and UV stabilizers was analyzed using only the SEC column. Fig. 2b shows the chromatogram obtained using only the silica column (without polymer), and Fig. 2c the chromatogram of the same solution when the whole system of Fig. 1 was applied.

Compared to the work of Johnson et al. [29] our procedure presents some advantages: (1) it is applied satisfactorily (only 30 min/analysis) to the determination of antioxidants and UV stabilizers, even if the resolution is low in some cases, as commented above; (2) no gradient is used, so that no equilibration time is needed, and a second HPLC gradient pump is unnecessary; (3) sensitivity is much higher than in the cited work because around 10 ml —the fraction between 9 and 20 min shown in Fig. 2a— are transferred to the second column instead of 10–50 μl in the previously commented case.

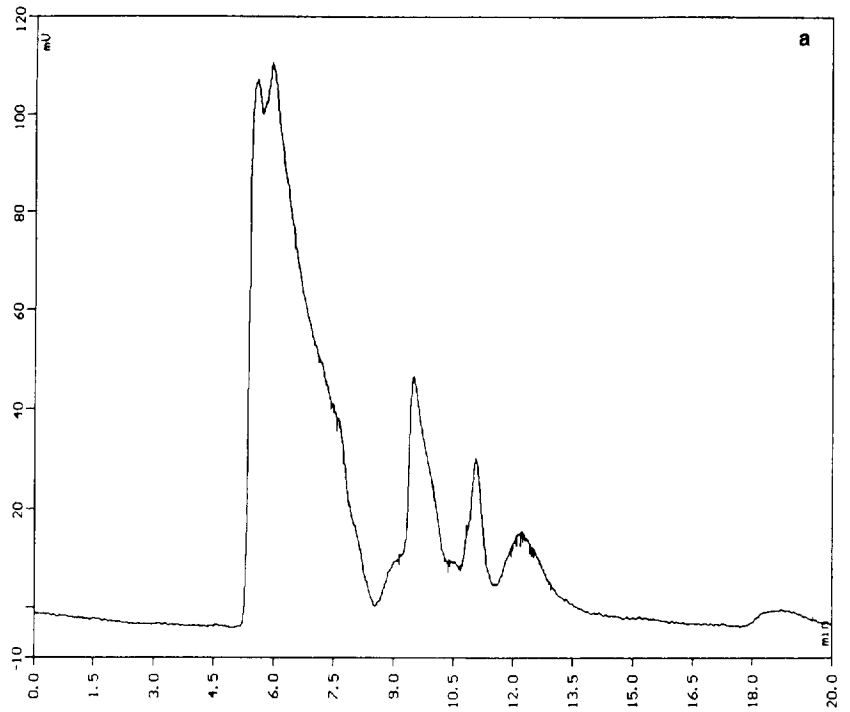
As can be seen, the separation of the compounds has been improved by the two columns in series. The polymer has been drained off through the valve and it is not introduced into the second column, so that the silica column is preserved from any damage and its operative life extended.

When the automatic valve changes from the drainage position to the normal-phase column position, the pressure of the system is slightly increased during a period of 30 s from 27 to 49 bar. This allows the drainage without problems. A 2-ml capillary loop was connected in the outlet of the valve in the drainage mode to simulate pressure of the system during the sudden break. Although the polarity gradient of solvent is supported by this system, the isocratic mode was used throughout to avoid long stabilization periods between different runs.

3.3. Analytical features

The detection limits, for all the compounds studied expressed as the equivalent concentration to three times the area of the background noise, are shown in Table 1. As can be seen, in all cases these values are about 0.1 μg ml^{-1} with the only exception of Cyasorb UV 1084 (because of its low sensitivity at the wavelengths used) and Cyasorb UV 9, because of its chemical nature (benzophenone derivative), has a very strong interaction with the stationary phase and a broad peak appears in the chromatogram.

The analysis of a standard solution containing all the mentioned compounds showed very good precision and accuracy, even in the case of peaks with incomplete separation, and quantitative accurate results were obtained (Table 1). A



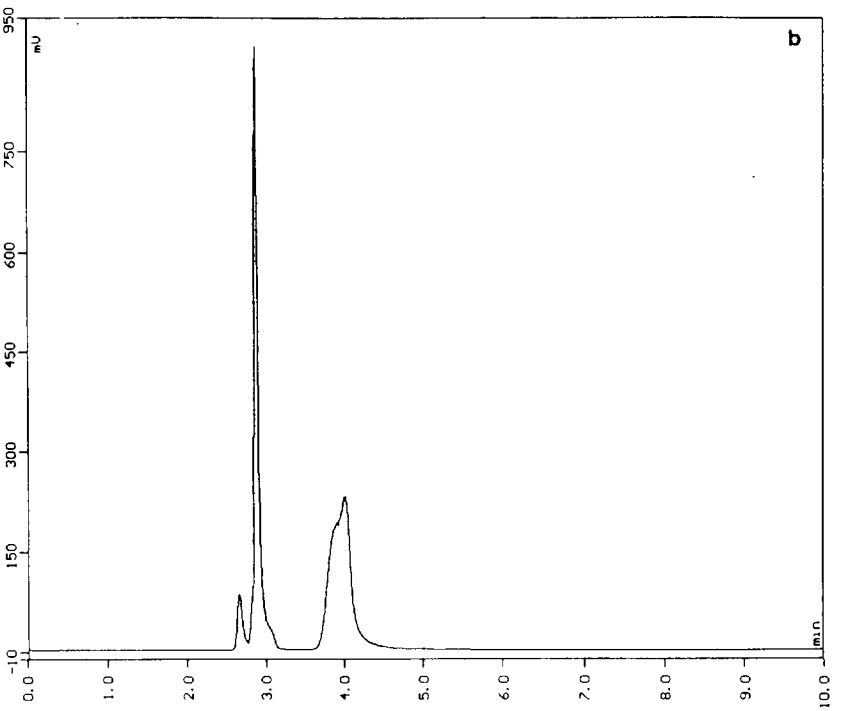


Fig. 2. (*Continued on p. 235*).

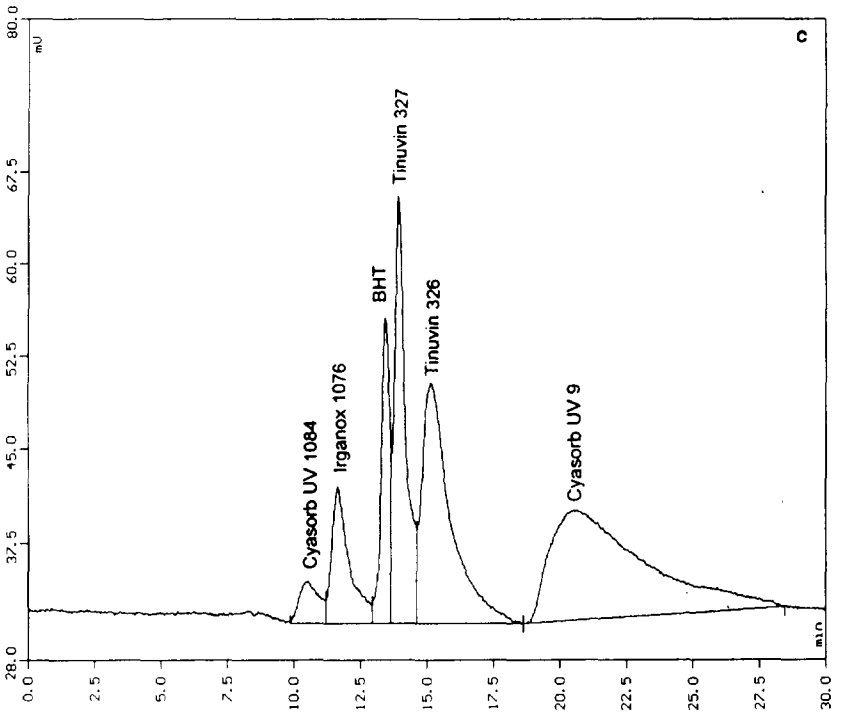


Fig. 2. Chromatograms obtained from (a) polymer (first peak) and additives only with SEC column and (b) additives only with normal-phase column. (c) Final chromatogram of the analysis of additives from polymer sample according to the proposed method. Conditions (for all cases): flow 0.9 ml min $^{-1}$; mobile phase *n*-hexane–dichloromethane (73:27, v/v); column temperature 35°C.

Table 1
Analytical characteristics of the determination of antioxidants and UV stabilizers by coupling both HPLC columns (SEC + normal phase)

Compound	Retention time (min)[a]	Detection limit (μg ml $^{-1}$)	Linearity (r)	Precision (R.S.D., %)[b]	Accuracy[a,c]	Linear range (μg ml^{-1})
Cyasorb UV 1084	0.50 ± 0.08	1.1	0.9989	3.2	24.4 ± 0.8 (24.8)	5.0–200
Irganox 1076	11.62 ± 0.07	0.2	0.9992	2.4	25.6 ± 0.6 (25.0)	1.0–200
BHT	13.44 ± 0.05	0.1	0.9994	3.7	25.4 ± 0.9 (24.9)	0.5–200
Tinuvin 327	13.95 ± 0.11	0.1	0.9981	4.0	24.1 ± 1.0 (24.8)	0.2–200
Tinuvin 326	15.12 ± 0.09	0.1	0.9978	2.9	25.8 ± 0.8 (25.3)	0.5–200
Cyasorb UV 9	20.64 ± 0.34	1.1	0.9976	4.2	26.0 ± 1.1 (25.5)	5.0–200

[a] Confidence level 95%, 6 replicates.
[b] Calculated with 6 replicates (ca. 25 μg ml $^{-1}$).
[c] Found value; real value in parentheses.

slightly higher sensitivity was obtained at 280 nm than at 254 nm. A wide linear range was obtained for all the compounds, which allows the direct determination of the compounds in the common plastics used.

4. Conclusions

The main conclusion is that on-line coupling of two high-resolution columns with different stationary phases —exclusion and adsorption— is an attractive and powerful methodology for the simultaneous analysis of different compounds by HPLC. So, this method permits the automatization of clean-up and analysis in one step, decreasing both sample handling and analysis time.

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