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Short communication

Feasibility of supercritical fluid extraction with on-line coupling of reversed-phase liquid chromatography for quantitative analysis of polymer additives

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Abstract

An on-line analytical method for determining polymer additives which incorporates (a) sample preparation and concentration, (b) chromatographic separation, and (c) UV detection is presented. The on-line system which combines supercritical fluid extraction (SFE) and high-performance liquid chromatography (LC) allows analytes to first be extracted and transferred to the SFE sorbent trap, then desorbed from the trap and presented to the LC system. No UV detector interference from residual dissolved CO_2 in the mobile phase was observed since CO_2 is eliminated by a pre-wash of the sorbent trap with a small amount of water. Reversed-phase LC with a mobile phase gradient of acetonitrile–water was employed. The described method allows a complete analysis to be processed in less than 30 min.

Keywords: Supercritical fluid extraction; Polymer additives

1. Introduction

There are few literature references regarding the direct coupling of supercritical fluid extraction (SFE) with high-performance liquid chromatography (LC). In most of these published methods, an undesirable low extraction flow-rate was required in order to efficiently trap the extracted analytes [1]. An impactor (packed sorbent bed) interface was usually employed to trap the analytes. By means of a switching valve, the extracted analytes could then be washed from the sorbent interface by mobile phase onto the head of an analytical column [2]. Quantitative data were generally missing from these early reports.

More recently, Ashraf-Khorassani et al. [3] have used on-line SFE–LC without intermediate trapping for analysis of PAHs. In their study, they injected a small fraction of the extracted analyte dissolved in supercritical CO_2 directly into the LC mobile phase. Since no intermediate trapping was performed, a significant peak was observed in the chromatographic trace with UV detection due to the presence of residual dissolved CO_2 in the mobile phase. In many cases, this undesirable peak obscured the detection of eluting analyte.

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Stone et al. [4] used a polymer-coated open tubular intermediate trap rather than a packed tube trap to connect SFE to LC. In this study, analytes were collected onto the coating of a small section of a megabore capillary column. These analytes were later washed via the LC mobile phase from the capillary trap onto the head of an analytical packed column. A major disadvantage in this approach was again that dissolved CO_2 in the mobile phase always created a huge peak with UV detection at the beginning of each separation which made the analysis long and likely obscured the elution of certain analytes. Extraction flow-rates in this study were much faster than previously reported because trapping was performed at 25 atmospheres in order to reduce the decompressed CO_2 flow-rate rather than under ambient conditions where the flow-rate would be considerably greater.

The purpose of our study was to use an existing commercial SFE system with addition of a single six-port two-position valve and an associated LC system to perform quantitative on-line SFE–LC without the nuisance of dissolved CO_2 in the mobile phase. We used a packed bed to trap extracted analyte using high flow-rates (2 ml/min) of liquid CO_2 and various percentages of methanol modifier in the extraction fluid. Analytes were polymer additives. The unique aspects of the work are (1) the quantitative nature of the results, (2) simplified interface without sacrificing optimal SFE or LC parameters, (3) extension to high modifier percentages, and (4) application to polymer additives/matrix.

2. Experimental

2.1. Instrumentation

An Isco-Suprex (Lincoln, NE, USA) AutoPrep-44 supercritical fluid extraction system equipped with Accutrap and modifier pump was used. All LC analyses were performed using an Agilent (Little Falls, DE, USA) model 1050 LC pump, UV detector, and autosampler. These extraction and chromatographic systems were interfaced via a Valco (Houston, TX, USA) six-port two-position valve. Accutrap was packed with stainless steel balls (for polymer additives). Polymer additive chromatographic separations were obtained with a Nova-Pak C_{18} column (150×3.9 mm, 5 μm d_p) from Waters (Milford, MA, USA).

2.2. SFE–LC details

The valve schematic for both the elimination of residual CO_2 and injection of extracted analyte into the LC is shown in Fig. 1. The SFE variable restrictor was heated to 60 °C for all extractions. The extraction trap (packed bed) temperature depended on analyte and extraction fluid composition. For volatile analytes, where 100% CO_2 could be used, trap temperature was set to 0 °C or lower, but for extractions using modified fluid, trap temperature was set at 40 °C or a higher temperature depending on the modifier concentration in order to prevent modifier condensation inside the trap during extraction. During the dynamic extraction period, the CO_2-containing extracted analytes were depressurized inside the heated restrictor and deposited on the cooled or heated packed trap during extraction. CO_2 gas was vented into a waste bottle filled with water.

After the extraction was completed, the dynamic/static valve was switched thus causing the CO_2 flow to be stopped. Next, the Accutrap trap rinse pump delivered water into the packed trap at a flow-rate of 1 ml/min thus filling the trap. Any excess water was flushed into the waste bottle. None of the extracted analytes were washed out of the trap when excess

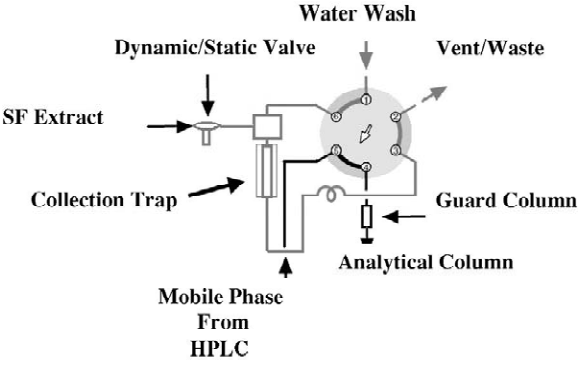

Fig. 1. Schematic of SFE–LC valve positions to achieve (1) elimination of residual CO_2. Rotate six-port valve one position to inject extracted analyte into LC.

water was transferred into the waste because of either the strong analyte/sorbent packing interaction or the low solubility of analyte in water. Next, a signal from the Suprex AP-44 to the LC system and six-port two-position interface valve was simultaneously transmitted to start the LC program and actuate the valve to the inject position. A guard column was, however, placed before the analytical column as a caution to prevent the escape of any particles from the solid-phase trap that may later plug the analytical column.

The interface was evaluated free of matrix effects with three polymer additives (Irganox 1010, Irganox 1076 and Irgafos 168). For each extraction, 5 μl of a standard solution (0.3 μg/μl/component) were spiked into an empty extraction vessel and then extracted. The SFE–LC interface was also applied to a polymethylmethacrylate (PMMA) sample which contained the same three polymer additives as previously mentioned. Coupled on-line data were compared with off-line SFE–LC data on the same PMMA sample. Table 1 shows the SFE and LC conditions that were used for extraction and analysis of the polymer additives.

3. Results and discussion

3.1. Demonstration of SFE–LC

The LC integrity of the polymer additives was first determined by comparing their separation when classically injected as a solution via syringe directly into the valve/LC column versus when the same volume of standard solution was first spiked into the packed solid-phase trap (e.g. make believe extraction/trapping) and then the trap was washed with (1) water to eliminate any residual gas inside the trap and (2) mobile phase to mobilize the analytes onto the column for chromatography. Both types of ''injections'' provided nice peak shapes although the resolution between peaks decreased slightly from extra column effects when the additives were first spiked into the extraction trap and then transferred onto the column for separation.

Next, we studied on-line SFE–LC of the same mixture wherein a solution of polymer additives was initially spiked into the extraction vessel, analytes extracted via pure CO_2, analytes collected on a trap of stainless steel balls at 0 °C, and subsequently the

Table 1
Extraction and chromatography conditions used for polymer additives[a]

Extraction parameters	
CO_2 extraction pressure	450 bar
Liquid CO_2 flow	2 ml/min
Modifier	None[b]—no matrix
Extraction temperature	80 °C
Static extraction time	5 min
Dynamic extraction time	25 min
Trap temp. during extraction	0 °C[b]
Trap temp. during rinse	25 °C
H_2O vol. passed through trap	4 ml
Trap packing material	Stainless steel balls
Trap dimensions	50×4.6 mm stainless steel
LC parameters	
Mobile phase program	80/20% acetonitrile–H_2O at t=0 min 100% acetonitrile at t=5 min hold for 15 min 80/20% acetonitrile–H_2O at t=21 min Equilibrate for 3 min
Column	Nova-Pak C_{18} column (150×3.9 mm, 5 μm d_p)
Flow-rate	2 ml/min
Detector	UV at 280 nm

[a] Five μl of the stock solution were placed in the empty extraction vessel.
[b] Polymer matrix: 10% methanol, trap temperature during extraction=80 °C, ground polymer.

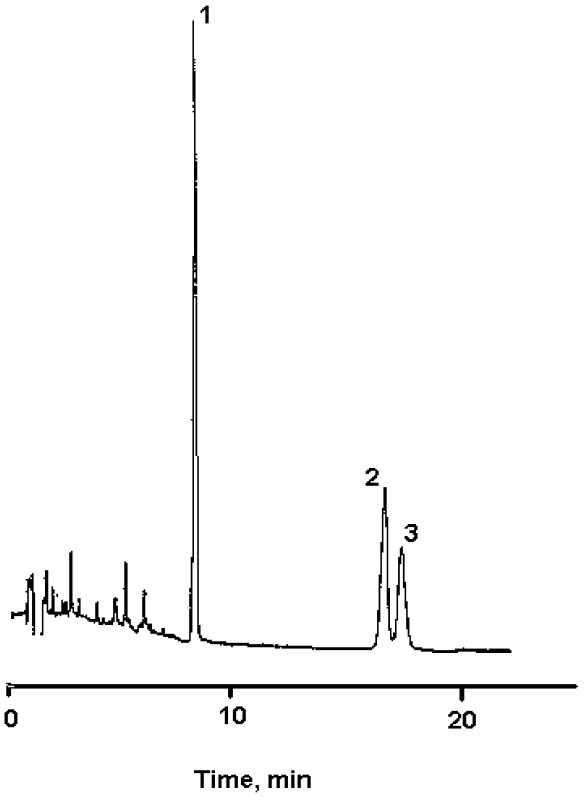

Fig. 2. SFE–LC of polymer additives spiked into open extraction vessel. (1) Irganox 1010, (2) Irganox 1076, and (3) Irgafos 168.

extracted analytes were analyzed via LC–UV, Fig. 2. At the beginning of the chromatography, the pressure of the LC pump quickly dropped from 103 bar to ~17 bar, but within a few seconds the pressure read ~159 bar. The initial pressure drop, we feel, was due to the presence of low pressure water in the trap which quickly was pressurized along with the mobile phase. The pressure rise above the initial (103 bar) pressure was caused by the flow of mobile phase with lower viscosity and density pushing water with higher viscosity and density through the analytical column. After 1–2 min, the LC pressure returned to its initial pressure. After several minutes into the LC run, the interface valve was returned to the load position which made it ready for extraction and collection of the next sample. The mobile phase composition was sufficient to wash all the extracted analytes from the sorbent trap onto the analytical column. Again, peak shapes and resolution between peaks for the mobile phase gradient acetonitrile–water reversed-phase separation were nearly as good as direct syringe injection onto the column.

For the SFE–LC interface to be truly useful, extractions with modified fluids must be feasible. Methanol is a common modifier for CO_2. Under sub-ambient trapping conditions, these modifiers are expected to be trapped along with analytes of interest thus causing the trap to become less efficient unless the trap can be raised in temperature to avoid modifier condensation. To determine the effect of CO_2 modifier concentration from the extraction and subsequent trapping on liquid chromatographic integrity, different CO_2 modifier concentrations were used as the extraction fluid. In order to prevent modifier condensation in the trap which previous experience suggests would result in loss of analyte, it was necessary to optimize the trap temperature prior to SFE–LC.

From Table 2, it can be observed that with pure CO_2 and a trap temperature of 0 °C, the extraction and collection efficiency of each polymer additive

Table 2
Effect of trap temperature and modifier concentration on percent recovery of polymer additives from spiked empty vessel using stainless steel balls as a trap material[a]

Extraction fluid (trap temp.)	Irganox 1010 (RSD)	Irganox 1076 (RSD)	Irgafos 168 (RSD)
100% CO_2 (0 °C)	94 (4)	98 (0.2)	97 (0.9)
95/5, CO_2/MeOH (40 °C)	100 (6)	104 (9)	101 (2)
100% CO_2 (60 °C)	96 (3)	98 (3)	98 (3)
90/10, CO_2/MeOH (60 °C)	73 (10)	76 (12)	76 (12)
90/10, CO_2 MeOH (80 °C)	90 (4)	90 (6)	93 (2)
90/10, CO_2/MeOH (90 °C)	97 (1)	99 (2)	99 (1)

[a] Each analysis represents the average of three individual extractions; 1.5 μg of each additive were spiked into the vessel.

(no matrix) was 95% or greater with RSD around 4.0% or less. Heating the trap to 60 °C seemingly had little effect on the recovery of each analyte with 100% CO_2 extraction. However, when the extraction fluid was modified to 95/5% CO_2/MeOH, as is many times the case with SFE of various matrices, it was necessary to heat the trap to 40 °C to have collection efficiencies greater than 99%. For extraction fluid modified with 10% methanol, it was necessary to heat the stainless steel bead trap to 90 °C in order to avoid methanol condensation on the trap and to achieve quantitative recovery. In general, our results showed that with increasing trap temperature not only was collection efficiency with modified fluids increased but RSD decreased for on-line SFE–LC just as was observed with off-line SFE followed by LC. It should be noted that for analysis of the neat polymer additives (i.e. no matrix) and the polymer additives in PMMA (vide infra), we were not able to use the more common adsorbent C_{18} packing for the extraction trap because considerable peak broadening and poor resolution for Irganox 1076 and Irgafos 168 were observed.

3.2. SFE–LC applied to polymer matrix

To test our SFE–LC interface on a more complex matrix and to compare our results with the off-line procedure, a 20% cross-linked PMMA sample prepared in-house and spiked before polymerization with 1000 μg/g of each additive was extracted using the off-line optimized SFE conditions that we had reported earlier [5]. In this case, acetone-modified CO_2 was employed rather than methanol-modified CO_2. A ground, unsieved sample of polymer (25 mg) was placed in the extraction vessel and SFE–LC was performed. Our results showed similar recovery of the Irganoxes with comparable precision relative to our earlier previously reported off-line study, Table 3. The disparity between on-line and off-line runs was, however, especially noticeable for Irgafos 168. Two different PMMA sample preparations were examined via the off-line method with similar results. Liquid–solid extraction (LSE) of the same PMMA preparations with methylene chloride yielded analogous results. In all three sample preparation (i.e. off-line SFE, on-line SFE, and LSE) protocols, the amount of additive found was smaller than had been incorporated into the polymer during synthesis (~20% Irganox 1010, ~70% Irganox 1076, ~90% Irgafos 168).

Since the sample size was 25 mg for the on-line measurement and 1000 mg for the off-line measurement, it was decided that the sample drawn for the on-line run may not have been representative of the bulk. The polymer sample was next sieved thereby yielding a uniform particle size. Table 3 shows our extraction recovery results before and after passing the ground sample through a fine mesh sieve. After sieving the sample and performing SFE–LC, not only was our RSD reduced relative to the unsieved data, but our recovery increased fourfold for Irganox 1010, 50% for Irganox 1076, and nearly threefold for Irgafos 168. We feel that the on-line results may more accurately reflect the true quantity of additive in the PMMA than the off-line data and LSE data since the on-line analysis conditions were more benign (e.g. lower temperature, absence of air). It should be noted that the chromatographic trace of the PMMA extract regardless of sample preparation

Table 3
Mass (μg) per gram of polymer additives extracted from PMMA as a function of sieving[a]

	Irganox 1010		Irganox 1076		Irganos 168	
	Not sieved	Sieved	Not sieved	Sieved	Not sieved	Sieved
On-line SFE–LC	186(12)	753(9)	680(12)	1078(9)	247(12)	719(9)
Off-line SFE–LC	242(9)[b]	–	515(2)	–	951(1)	–
	314(3)[c]		582(8)		1064(7)	
Liquid–solid ext.	242[b]	–	705	–	1279	–
(CH_2Cl_2)+LC	263[c]		448		912	

[a] Numbers in parentheses are RSD (n=4).
[b] First preparation of in-house sample.
[c] Second preparation of in-house sample.

Fig. 3. SFE–LC of polymer additives (1000 μg/g each additive) from 20% cross linked PMMA prepared in-house, ground 2×, and sieved. See Table 1 for chromatographic conditions.

method suggested that the co-extraction of polymer oligomers had also occurred, Fig. 3.

4. Conclusion

In summary, a more user-friendly interface than previously reported for the direct coupling of SFE and reversed-phase LC has been quantitatively evaluated. The operating parameters of SFE and RP-LC are not compromised (as in previous reports) by having them interfaced to each other. Chromatographic and extraction/trapping efficiency were maintained. The experiment was applied to mixtures of polymer additives. Our analysis of polymer additives in PMMA may be more accurate via the on-line method because the analytes are extracted at relatively low temperature in the absence of light and under anaerobic conditions.

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