

**Quantitative Analysis of Additives in Low Density Polyethylene Using On-line
Supercritical Fluid Extraction /Supercritical Fluid Chromatography**

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(ABSTRACT)

Polymer additives exemplify many classes of compounds which possess a wide variety of chemical (i.e., phenols, amides, esters) and physical (i.e., volatility, solubility) properties. They are incorporated into polyolefins and other such polymeric materials for a number reasons: (a) to prevent degradation by ultraviolet light, heat, and oxygen; (b) to aid in the processing of the polymer; and (c) to modify the physical properties of the polymer. Since the purity and amount of additive can affect polymer properties, it is very important to characterize and quantify additives in polymer products. Traditional liquid solvent/polymer extraction methods, which involve dissolution/precipitation, are time-consuming, uneconomical, and the recoveries are significantly lower than 90%.

In recent years, analysis with supercritical fluids (SFs) has emerged as an alternative analytical technique because SFs afford higher diffusivity and lower viscosity. In this research, an on-line Supercritical Fluid Extraction (SFE)/Supercritical Fluid Chromatography (SFC) system was assembled to provide efficient extraction and separation of polymer additives with quantitative results. The effects of various SFE/SFC parameters, such as trapping temperature, injection temperature, extraction pressure and temperature, dynamic extraction time, and fluid flow rate on extraction and separation efficiencies of different additive standards (i.e., BHT, BHEB, Isonox 129, Irganox 1076 and Irganox 1010) were investigated. Optimized conditions were employed to quantitatively extract additives from LDPE. Identification of additives was performed by comparing the retention time with each additive standard. Results obtained from on-line SFE/SFC were compared to results from off-line SFE/High Performance Liquid Chromatography (HPLC) and off-line Enhanced Solvent Extraction (ESE)/HPLC.

This thesis is dedicated to
Haiqing, Amy, Dad, Mom, and Tong

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Chapter 1

Introduction

Extraction and Separation with Supercritical Fluids

Supercritical Fluids

A typical phase diagram for a pure substance (**Figure 1.1**) shows the temperature and pressure region where the substance occurs as a single phase [viz., solid (s), liquid (l) or gas (g)]. There are three curves describing the sublimation, melting and boiling processes. The three curves intersect at the so-called triple point (TP), where the solid, liquid, and gaseous phases coexist in equilibrium. Points along the curves (between the phases) define the equilibrium between two of the phases. The boiling curve starts at the TP and ends at the critical point (CP). The critical pressure (P_c) is defined as the maximum pressure at which a liquid can be converted to a gas by an increase in temperature. Whereas the critical temperature (T_c) is the highest temperature at which a gas can be converted to a liquid by an increase in pressure. We can therefore define a supercritical fluid (SF) as any substance that is above its critical pressure and critical temperature. The region of pressures and temperatures above P_c and T_c is called the supercritical region ¹.

The critical point is characteristic for each substance. **Table 1.1** ² lists the critical pressure and temperature for various solvents, as well as the fluid density at the critical point, which is called the critical density (ρ_c) ¹. By far the most common fluid used in supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC) is carbon dioxide (CO_2). The critical parameters of CO_2 are about 31°C and 73 atm, which are easily obtained in the laboratory. In addition, CO_2 is nonflammable, nontoxic, less expensive than reagent grade liquid solvents, readily available in a high state of purity, and environmentally friendly ¹.

¹ Taylor L.T. (1996) Supercritical Fluid Extraction, John Wiley & Sons, Inc., New York

² Luque de Castro M.D., Valcarcel M., Tena M.T. (1994) Analytical Supercritical Fluid Extraction, Springer-Verlag, Berlin

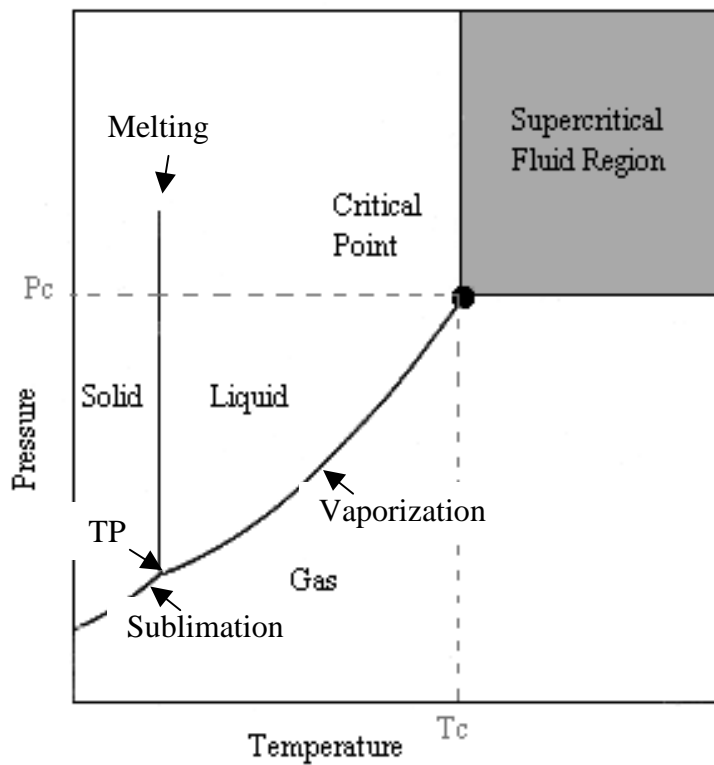


Figure 1.1. Solid-liquid-gas-supercritical fluid phase diagram. (Taken from ref. 1)

Table 1.1. Features of various solvents at the critical point. (Taken from ref. 2)

Solvents	T_c (°C)	P_c (bar)	ρ_c (g/mL)
Inorganic			
Carbon dioxide	31.1	72	0.47
Dinitrogen monoxide	36.5	70.6	0.45
Ammonia	132.5	109.8	0.23
Water	374.2	214.8	0.32
Sulfur hexafluoride	45.5	38	
Helium	-268	2.2	0.07
Xenon	17	56.9	1.11
Hydrocarbons			
Methane	-82	46	0.169
Propane	96.7	42.4	0.22
Ethylene	11	50.5	0.2
Benzene	288.9	98.7	0.302
Toluene	319	41.1	0.292
Alcohols			
Methanol	239	78.9	0.27
Isopropyl alcohol	235.3	47.6	0.273
Ethers			
Ethyl methyl ether	164.7	47.6	0.272
Tetrahydrofuran	267	50.5	0.32
Halides			
Trifluoromethane	26	46.9	0.52
Dichlorodifluoromethane	111.7	109.8	0.558
Chlorotrifluoromethane	28.8	214.8	0.58
Trichlorofluoromethane	196.6	28.9	0.554
Miscellaneous			
Acetonitrile	275	47	0.25
Pyridine	347	56.3	0.312

A SF exhibits physicochemical properties intermediate between those of a liquid and a gas. The physical properties of a gas, liquid and SF are compared in **Table 1.2**¹. The density of a SF which is always close to the typical values for liquids, depends on the pressure and temperature to which it is subjected. The high density is responsible for the good solvating power of SFs, where interactions between the fluid and solute molecules are quite strong. SFs have more favorable hydrodynamic properties than those of liquids because supercritical viscosity values are more like those of gases¹. On the other hand, their near-zero surface tension allows them to readily penetrate porous solids and packed beds. For constant column dimensions, the pressure drop along an SFC column is typically ten times smaller than it is in liquid chromatography (LC), however, ten times greater than in gas chromatography (GC)³. The diffusion coefficients of solutes in SFs are between those displayed for liquids and gases. Mass transfer relative to a liquid is rapid in SFs because diffusivities of SFs are higher than those of liquids¹. In conclusion, the properties of gas-like diffusivity and viscosity, coupled with liquid-like density, combined with the pressure and temperature-dependent solvating power of SFs lead to more expeditious and efficient analytical extraction and separation.

Supercritical Fluid Extraction

Figure 1.2 shows a schematic diagram illustrating the basic elements of a supercritical fluid extraction (SFE)². A gas cylinder provides a source of SF (e.g., CO₂). Both syringe and reciprocating pumps can be used as solvent delivery systems. For the instrumentation used in this thesis, a syringe pump was employed. Although syringe pumps are relatively expensive, they deliver pulse-free flow over a large range of flow rates¹. A supplementary modifier pump is used if the analyte/matrix to be extracted requires a polar modifier. Stainless steel or fused silica tubing is used to connect the various parts of the extraction apparatus.

The extraction chamber or vessel is the compartment where the sample is placed

³ Hawthorne S.B., Miller D.J., Langenfeld J.J. (1990) *J. Chromatogr. Sci.* **28**, 2

Table 1.2. Comparison of the physical properties of supercritical CO₂ and those of ordinary gases and liquids. (Taken from ref. 1)

	Density (g/cm ³)	Viscosity (g/cm·s)	Diffusion coefficient (cm ² /s)
Gases	0.0001-0.002	0.0001-0.0003	0.1-0.4
Supercritical CO ₂ T _c , P _c	0.47	0.0003	0.0007
T _c , 6P _c	1.0	0.001	0.0002
Liquids	0.6-1.6	0.002-0.03	0.000002-0.00002

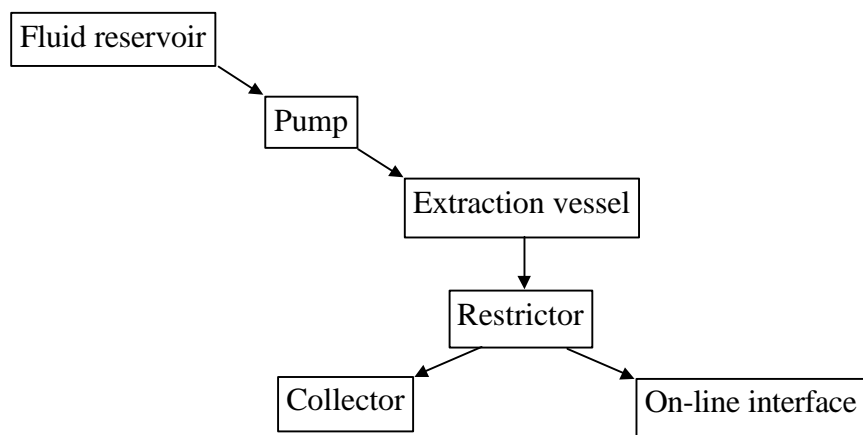


Figure 1.2. Basic scheme of SFE system. (Taken from ref. 2)

for subjection to the action of the SF. It must be capable of withstanding high pressure (300-600 atm)¹. The extraction vessel is usually a stainless steel cylinder of varying length and inner diameter. The high pressure rating and the absence of leaks are characteristic of SFE vessels¹. The vessel is in turn placed in a temperature-controlled zone, which is required, since the critical temperature of most SFs is above room temperature.

The pressure change from supercritical conditions in the extraction vessel to the prevailing atmospheric conditions is effected via an interface known as a restrictor. Commercially available restrictors are of two types: fixed restrictors, which are manufactured in various designs (e.g., linear, tapered, integral, pinhole, and frit), and variable restrictors¹. Heating of the restrictor is usually required to avoid plugging through freezing. In the on-line SFE/SFC system used in this research, a linear fused silica capillary was employed as a vessel outlet restrictor.

Following the restrictor is a trapping device. There are three basic types of SFE systems characterized by the way in which the solutes are isolated from the SFE media used⁴. In the first type, solutes are separated from the extraction media based on pressure reduction, which causes a solubility decrease. In the second type, a temperature change is used to bring about a decrease in solubility from the extraction media, and in the third type solutes are absorbed onto an appropriate adsorbate. Often a combination of the first and second types is used, where after extraction the SF is simply evaporated to leave the solutes of interest. The simplest way of collection is when the restrictor outlet is inserted through the septum of a collection vial containing a few milliliters of solvent. The most common way of collection is solid phase trapping. The materials used for this purpose are column packings or inert surfaces. The solid phase trapping system is often heated or cooled depending on the volatility of the target analytes. In any case, this collection mode involves an additional step which is desorption of the analytes from the adsorbent by elution with a small amount of solvent for subsequent analysis or, alternatively, thermal

⁴ Saito M., Hondo T., Yamauchi Y. (1988) Fractionation by coupled micro-supercritical fluid extraction and supercritical fluid chromatography, Supercritical Fluid Chromatography, ed. Smith R.M., RSC Chemistry Monographs, London

desorption and sweeping of the trap by the eluent if an on-line coupled system is used. The trapping temperature depends on whether the analytes are to be isolated from the fluid. The collection chamber should be sealed in order to avoid losses of the analytes. In this research, a cryogenic trap served as the interface between SFE and SFC. Thermal desorption and sweeping the trap with SF CO₂ was employed to flush analytes onto the SFC column.

Contact between the SF and sample from which extraction takes place can be established in a static or dynamic mode¹. In a static extraction, the sample matrix is soaked in a fixed amount of SF. This type of extraction is often compared to a teabag in a cup of water. In a dynamic extraction, SF continuously passes through the sample matrix. This is analogous to a coffee maker¹. Typically a dynamic extraction can be more exhaustive than a static extraction. SFE can be performed in the dynamic mode, static mode or a combination of the two.

In order to develop an efficient and quantitative extraction method, many experimental parameters must be optimized. The extraction pressure is an important variable because the density, and hence the solvating power of SF is directly related to the pressure. The effect of temperature is more complicated than that of pressure. Increasing the temperature increases the diffusion coefficients of the solutes, whereas at the same time it also decreases the density. In addition, the considerations of fluid flow rate, addition of a modifier, and extraction time should be explored to achieve highest recoveries.

Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) may be defined as a form of chromatography (i.e., a physical separation method based on partitioning of an analyte between the mobile phase and the stationary phase) in which the mobile phase is subjected to pressures and temperatures near or above the critical point for the purpose of enhancing

the mobile phase solvating power ⁵. A schematic of a SFC system is shown in **Figure 1.3** ⁵. The use of SFs as chromatographic mobile phases was first reported in 1962 by Klesper, Corwin and Turner ⁶. However, early development in this field was slow due to experimental problems in using SFs, the lack of commercially available SFC instrumentation, and its being overshadowed by the simultaneous growth of high performance liquid chromatography (HPLC) ⁷. The recent resurgence of interest in SFC is due to the potential advantages afforded by the unique characteristics of the mobile phase in SFC over GC and HPLC, and more importantly, it has been augmented by increased technology in pumps and detectors for SFC.

The best way to illustrate these attributes of SFC is a direct comparison with HPLC. **Figure 1.4** shows Van Deemter plots for HPLC and SFC on the same packed column under the same operating temperature ⁸. The Figure indicates that higher analyte diffusivity results in higher optimum average linear velocities (μ_{opt}) for SFs than for liquids, which results in increased speeds of analysis for SFC as compared to HPLC. When compared to HPLC, higher analyte diffusivity causes narrower chromatographic peaks, which results in increased detector sensitivity ^{8,9}.

Lower viscosity of SFs causes a lower column pressure drop across a SFC column than that observed using the same column for HPLC. Therefore longer packed columns can be possibly used in SFC to increase total efficiency.

GC-like open-tubular columns have been also used in SFC. The bonded phases of SFC open tubular capillary columns typically are cross-linked more than are those of GC columns. This protects the stationary phase from being stripped by the harsher supercritical mobile phases. The use of capillary columns has advantages over packed

⁵ Lee M.L., Markides K.E. (1990) Analytical Supercritical Fluid Chromatography and Extraction, ed. Chromatography Conferences, Inc., Provo, Utah

⁶ Klesper E., Corwin A.H., Turner D.A. (1962) *J. Org. Chem.* **27**, 700

⁷ Novotny M. (1981) *Chromatographia* **14**, 679

⁸ Gere D.R. (1983) *Science* **222**, 253

⁹ Gere D.R. (1983) Assay of caffeine in beverages by supercritical fluid chromatography, *Hewlett-Packard Publication* No. 43-5953-1695, 1-6

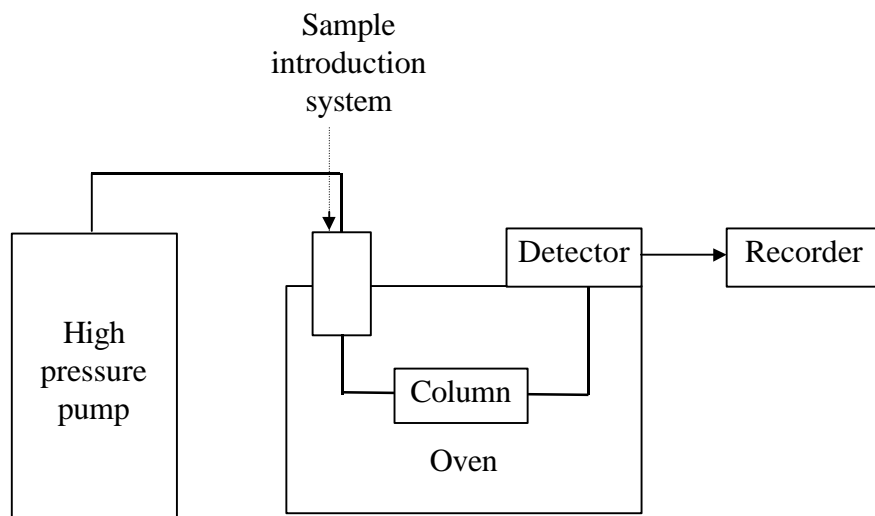


Figure 1.3. Schematic diagram of SFC system. (Taken from ref. 5)

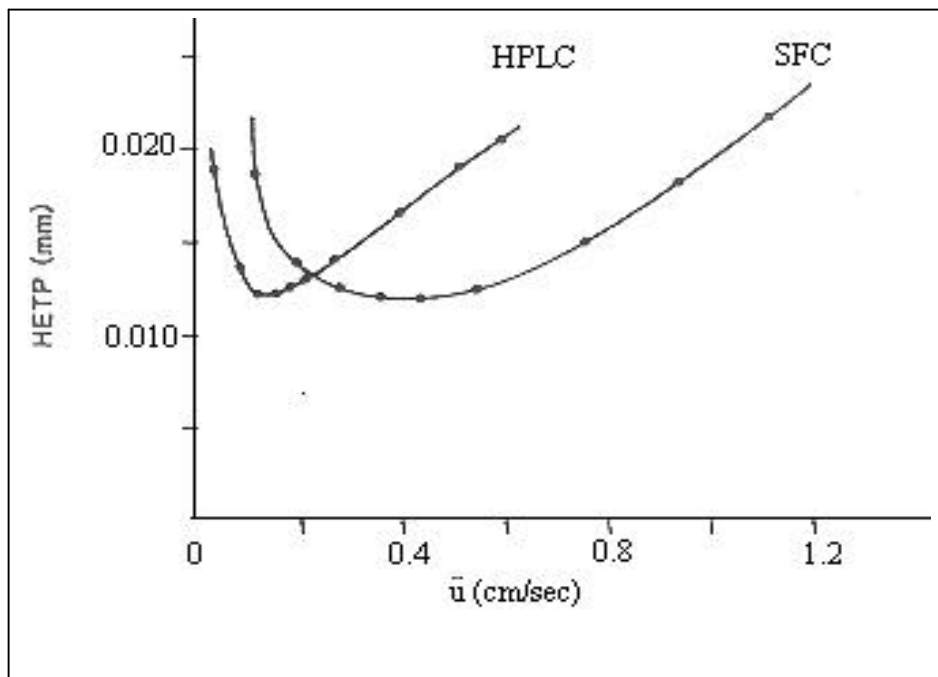


Figure 1.4. Van Deemter plots for chromatographic data from SFC and HPLC elution of pyrene. (Taken from ref. 8)

The plate height measurements were made using the same ODS column, the same operating temperature (40°C), and the same solute (pyrene). The mobile phase for the HPLC separation was acetonitrile:water (70:30 by volume); the mobile phase for the SFC separation was carbon dioxide at the average density 0.75 g/cm³. These conditions provide approximately the same capacity factor (k) for pyrene in both cases.

columns in SFC just as in GC. Capillary columns generally produce sharper chromatographic peaks than packed columns, which results in improved separation and detector sensitivity. Potentially, the main advantages capillary column SFC has over packed column SFC is that longer capillary columns can be used. Thus, the separations using capillary columns can be performed using a greater number of theoretical plates, i.e., increased efficiency. The low pressure drop and open-tubular nature of the capillary allow very long columns (e.g., 60 m) to be used. Packed columns, however, can handle higher sample-loading capacity, which can yield lower detection limits and higher flow rates, thus shortening analysis time.

Density and density gradients control elution in SFC where both pressure and temperature can be adjusted to obtain the desired densities. This is an advantage over GC methodology in which temperature is virtually the only adjustable parameter. A wide range of densities in SFC yields greater mobile phase solvation than do the inert gases used in GC. SFC does not suffer from volatility limitations as GC does. SFC is a particularly good technique for analysis of thermally labile, reactive or involatile materials, because separations are performed at low temperatures. In addition, SFC mobile phases can be modified to enhance solvating power.

One of the principal benefits of SFC is the flexibility of using both GC and HPLC detectors. For inorganic mobile phases such as CO₂, ammonia, and xenon, a universal flame ionization detector (FID) commonly is chosen. However, organically modified SFC mobile phases must be avoided with this detector. Other GC detectors commonly applied to SFC include nitrogen, phosphorous, flame photometric, Fourier transform-infrared (FT-IR) spectrometric, and mass spectrometric (MS). Many LC detectors are useful for mobile phases that are incompatible with the GC type detectors. Ultra-violet (UV) absorbance, fluorescence, and refractive index (RI) detectors are compatible with SFC.

On-line Supercritical Fluid Extraction /Supercritical Fluid Chromatography

SFE is generally not selective enough to isolate specific solutes from the matrix without further clean-up or resolution from co-extracted species prior to qualitative and quantitative analysis. Consequently, for analytical applications, SFE is usually used in conjunction with chromatographic techniques, to improve the overall selectivity of the process in isolating specific solutes. SFE combined with chromatography can be either “off-line” or “on-line”. In the off-line process, SFE takes place as a separate and isolated process to the chromatography. Whereas in the on-line process, SFE and chromatography are coupled to form an integrated process. In other words, the extracted species are passed directly to the chromatograph, usually via a trap or sample loop and a valve-switching device.

Among all these coupling techniques, on-line SFE/SFC is the most feasible combination. One obvious advantage is that the solvent used to inject the sample on the column is the same as the mobile phase¹⁰, the primary requisite for effective coupling of two techniques (viz., compatibility between the output of the first system and input of the second) is met. The first on-line SFE/SFC system was introduced by Sugiyama *et al.*¹¹ and, separately, Skelton *et al.*¹². Sugiyama *et al.* investigated a direct coupling of SFE to SFC through two six-way valves, a injector valves, and an extract trap loop. They demonstrated that the on-line system allowed the analyst to apply raw and/or solid samples to the system to obtain chromatogram of sample extracts. Skelton *et al.* described an alternative method whereby both extraction of the sample and introduction of the extract onto the column was accomplished on-line using only the supercritical fluid mobile phase. This sampling technique was made possible by a simple valving scheme which ties directly the extraction vessel, the injector, the packed column and the detector. Different samples (e.g., coal and coffee) were conducted with this on-line valving scheme, giving

¹⁰ Jackson W.P., Markides K.E., Lee M.L. (1986) *J. High Resolut. Chromatogr. Chromatogr. Commun.* **9**, 213.

¹¹ Sugiyama K., Saito M., Hondo T., Senda M. (1985) *J. Chromatogr.* **332**, 107

¹² Skelton R.J. Jr., Johnson C.C., Taylor L.T. (1986) *Chromatographia* **21**, 3

the results similar to the off-line, traditional methods. The efficient, fast and selective extraction capabilities of SFs allow quantitative extraction and direct transfer of selected solutes to the column, often without the need for further sample treatment or clean-up.

In addition to the above, major advantages of on-line SFE/SFC are enhanced capabilities that are normally beyond the scope of either technique when used separately. These enhanced capabilities include 1) suitability for trace analysis, 2) sample preparation with minimal sample contamination, 3) the ability to rapidly extract and directly analyze unstable and oxidation sensitive solutes, and 4) on-line automation of the sample preparation step with the chromatographic analysis step¹³.

The on-line SFE/SFC is nearly always achieved through the on-line linkage of a SFE vessel through a valve switching system, although other means for the direct introduction of SFE extracts such as the thermal modulation interface, developed by Mitra and Wilson¹⁴, have been used for capillary SFC. **Figure 1.5** shows the configuration for the on-line SFE/SFC system used in our work. The switching valves allow the extracted sample to be collected in a cryogenic accumulator while the same pump delivers the SF. The SF acts as the mobile phase for chromatography and the extracts are focused on the cryogenic trapping interface located prior to the chromatographic column. The purpose of the cryofocusing is to concentrate the solutes of interest into a narrow band for “injection” onto the SFC, leading to better detection limits, easier quantitation and more reproducibility^{13,15-16}. After extraction and collection, the valves are actuated and the trap temperature ballistically raised by means of an external heating system. In this way, the SF transfers the extracted substances from the trap to the analytical column.

¹³ Ashraf-Khorassani M., Levy J.M. (1990) *J. High Resolut. Chromatogr.* **13**,742

¹⁴ Mitra S., Wilson N.K. (1990) *J. Chromatogr.Sci.* **28**,182

¹⁵ Xie Q.L., Markides K.E., Lee M.L. (1989) *J. Chromatogr.Sci.* **27**,365

¹⁶ Yocklovich S.G., Saner S.F., Levy E.J. (1989) *Amer. Lab* **5**, 26

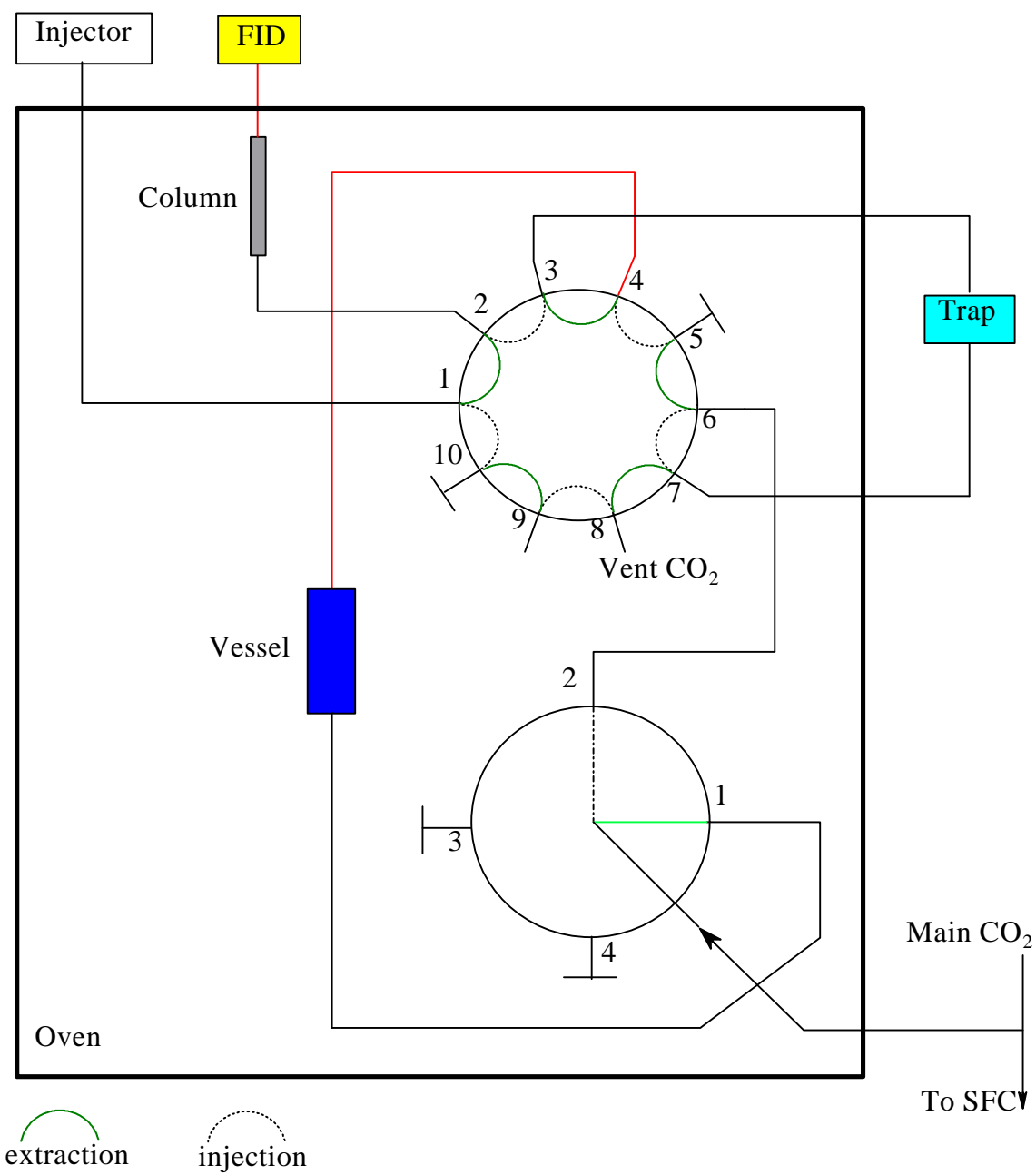


Figure 1.5. Schematic diagram of on-line SFE/SFC used in our study.

Polymer Additives

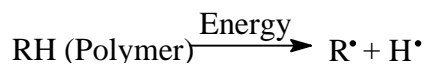
Polymer Additives

Since the early stages of the development of the polymer industry, it was realized that useful products could only be obtained if certain additives were incorporated into the polymer matrix¹⁷. The term “additive” is used here to describe those materials, which are physically dispersed in a polymer matrix without affecting significantly the molecular structure of the polymer¹⁷. **Table 1.3** shows the convenient classification of additives into groups and subdivision according to their more precise functions.

The factor which most of all determines product quality and output in polymer processing is resistance of the polymer to thermal degradation. The degradative effects of oxidation can be so devastating that without the presence of antioxidants, some polymers are rendered completely useless¹⁷.

Before discussing antioxidants, however, let us look more closely at the oxidative degradation process. The term “degradation” is used here to denote any chemical process, which alters the chemical structure of the polymer in a manner that leads to a deterioration in its physical properties¹⁷. Polymeric degradation brought about by the effects of heat, oxygen, mechanical shearing, or radiation typically occurs via a free radical mechanism¹⁸.

(1) Initiation step: Production of free radicals



It can occur in any one of the various phases of a polymer’s life circle: polymerization, processing, and end use.

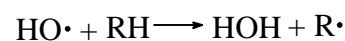
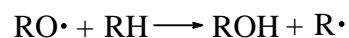
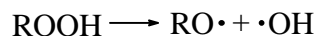
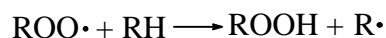
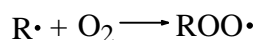
(2) Propagation step: Radicals interact with polymer chains

¹⁷ Mascia L. (1974) The Role of Additives in Plastics, Edward Arnold Ltd., London

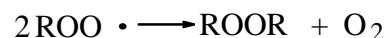
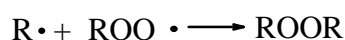
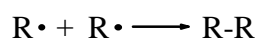
¹⁸ Lutz J.T. Jr. (1989) Thermoplastic Polymer Additives, ed., Marcel Dekker, Inc., New York and Basel

Table 1.3. Classification of additives. (Taken from ref.17)

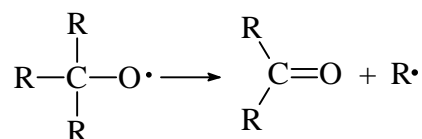
Group	Function
i) Additives which assist processing	<ol style="list-style-type: none">1) Processing stabilizer2) Lubricants (internal, external)3) Processing aids and flow promoters4) Thixotropic agents
ii) Additives which modify the bulk mechanical properties	<ol style="list-style-type: none">1) Plasticizers or flexibilizers2) Reinforcing fillers3) Toughening agents
iii) Additives used to reduce formulation	<ol style="list-style-type: none">1) Particulate fillers2) Diluents and extenders
iv) Surface property modifiers	<ol style="list-style-type: none">1) Antistatic agents2) Slip additives3) Anti-wear additives4) Anti-block additives5) Adhesion promoters
v) Optical property modifiers	<ol style="list-style-type: none">1) Pigments and dyes2) Nucleating agents
vi) Anti-aging additives	<ol style="list-style-type: none">1) Anti-oxidants2) UV stabilizers3) Fungicides
vii) Miscellaneous	<ol style="list-style-type: none">1) Blowing agents2) Flame retardants



(3) Termination step: Deactivation of free radicals



The last step above represents crosslinking, which increases the molecular weight of the polymer; this type of degradation manifests itself as brittleness, gelation, and decreased elongation.



The above represents chain scission, which results in a decrease in molecular weight, leading to increased melt flow and reduced tensile strength.

Antioxidants do not completely eliminate oxidative degradation, but they markedly retard the rate of autoxidation by interfering with radical propagation¹⁸. Two general classifications can be used to categorize antioxidants: primary (chain terminating) and secondary (peroxide decomposing). A list of major commercial antioxidants is given in **Table 1.4**, and some of the phenolic chemical structures are given in **Figure 1.6**¹⁸.

Hindered phenols and secondary arylamines act as primary antioxidants by donating their reactive hydrogen (N-H, O-H) to propagating free radicals, particularly peroxy radicals and thus form non-reactive products.

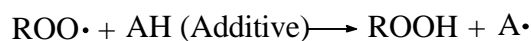
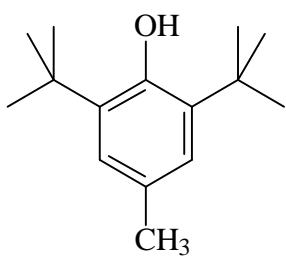
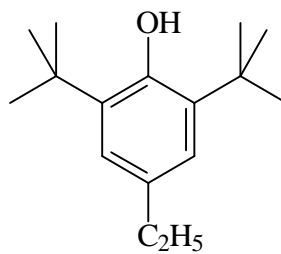


Table 1.4. Major commercial antioxidants. (Taken from Ref. 18)

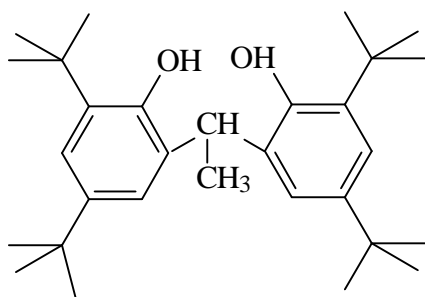
Registered trade name	Chemical name	MW	Physical form	Supplier
Phenolics				
BHT	2,6-di-t-butyl-4-methylphenol	220	Solid	Various
BHEB	2,6-di-t-butyl-4-ethylphenol	234	Solid	Various
Isonox 132 Vanox 1320	2,6-di-t-butyl-4-sec-butylphenol	262	Liquid	Neville Chemical Company Schenectady R.T.Vanderbilt
Cyanox 425	2,2'-methylenebis(4-ethyl-6-t-butylphenol)	368.5	Solid	American Cyanamid
Isonox 129 Vanox 1290	2,2'-Ethylidenebis-(4,6-di-t-butylphenol)	438	Solid	Schenectady R.T. Vanderbilt
Irganox 1076 Naugard 76 Oxi-Chek 116	Octadecyl 3,5-di-t-butyl-4-hydroxyhydrocinnamate	531	Solid	Ciba-Geigy Uniroyal-Chemical Ferro
Irganox 1010	Terakis [methylene-3-(3,5-di-t-butyl-4-hydroxyphenyl) propionate] methane	1178	Solid	Ciba-Geigy
Amines				
Wingstay 29 Vulkanox	p-oriented styrenated diphenylamine	320	Liquid/ Solid	Goodyear Mobay
Agerite DPPD Naugard J Permanax DPPD	N,N'-diphenyl-p-phenylene-diamine	260	Solid	R.T. Vanderbilt Uniroyal Chemical Vulnax
Thioesters				
Cyanox 711 Argus DTDTDP Evastab 13	Ditridecyl thiodipropionate	543	Liquid	American Cyanamid Argus Evans
Seenox 412-S	Pentaerythritoltetrakis [3-(dodecylthio)propionate	1162	Solid	Argus
Phosphites				
Weston 618 Mark 5060	Distearyl pentaerythritol diphosphite	732	Solid	Sandoz
Ultranox 626	Bis(2,4-di-t-butyl) pentaerythritol diphosphite	604	Solid	Borg-Warner



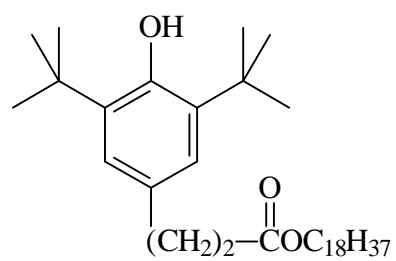
BHT



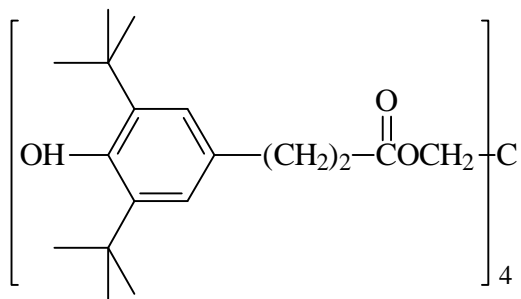
BHEB



Isonox 129



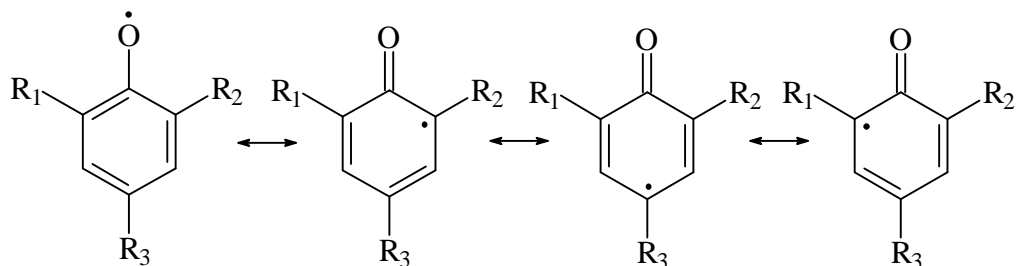
Irganox 1076



Irganox 1010

Figure 1.6. Chemical structures of hindered phenols.

This mechanism holds mainly for phenol derivatives; in these cases the inactive radical (A·) is stabilized by resonance:



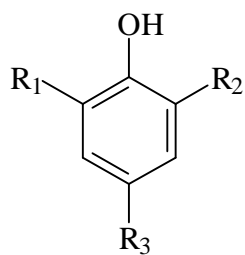
This mechanism has not been well established for arylamines, except in a few cases¹⁸.

Hindered phenolics are the more preferred type of primary antioxidants for thermoplastics. This group can be further categorized into the forms illustrated in **Figure 1.7**: (1) simple phenolics, (2) bis-phenolics, (3) polyphenolics, and (4) thiobisphenolics¹⁸.

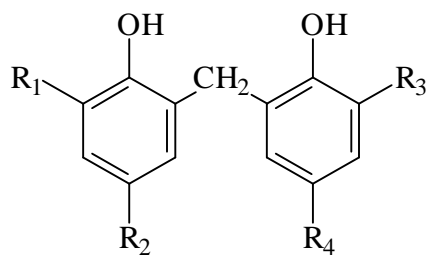
The most familiar hindered phenol, by far, is 2,6-di-*t*-butyl-4-methylphenol, also widely known by its trade name, BHT. **Figure 1.8** shows the chain terminating mechanism of BHT¹⁸.

Secondary antioxidants are also termed preventive stabilizers, because they prevent the proliferation of alkoxy and hydroxy radicals by decomposing hydroperoxides¹⁷. Many sulfur and phosphorous compounds can in fact act as secondary antioxidants and the most notable types are organophosphites and thioesters (**Table 1.4**). The mechanism of their reaction is complex but there seems to be some agreement that peroxides are reduced to alcohols and are, therefore, deactivated in the manner shown in **Figure 1.9**¹⁷.

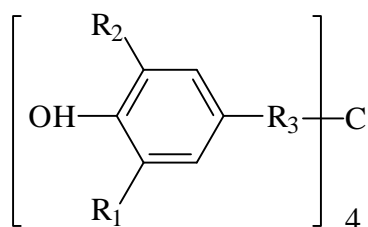
There are also other types of additives (**Table 1.3**) incorporated within polymers, such as antiblocking agents. Antiblocks are necessary to avoid the polymer film sticking to itself as a result of storage in roll form. Typical antiblocks can be silica, talc, or diatomaceous earth.



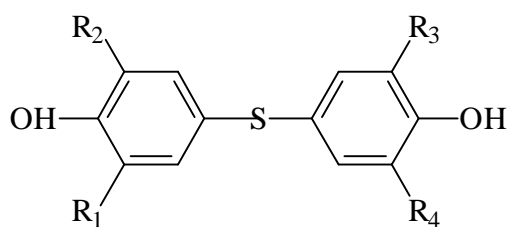
(1)



(2)



(3)



(4)

Figure 1.7. Hindered phenolic types: (1) simple phenolics, (2) bis-phenolics, (3) polyphenolics, and (4) thiobisphenolics. (Taken from ref. 18)

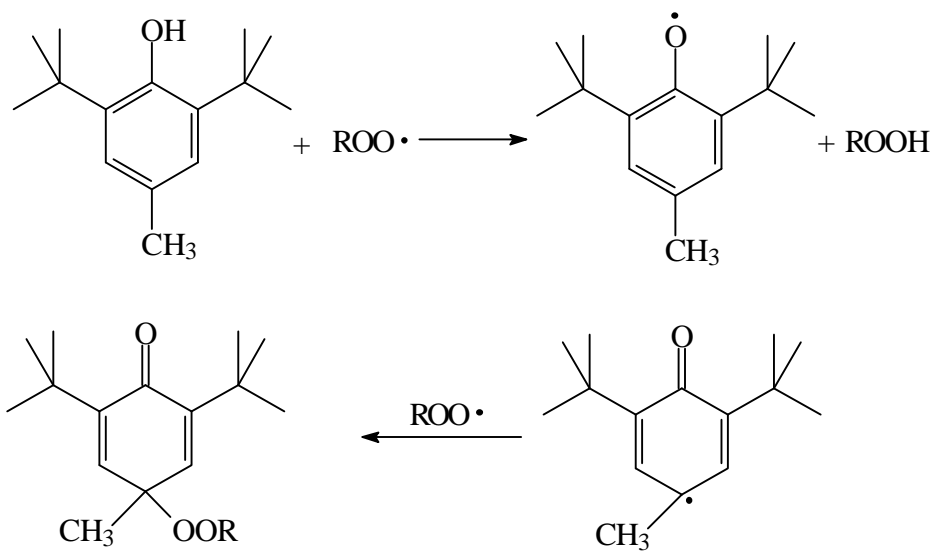


Figure 1.8. The chain terminating mechanism of BHT, a hindered phenolic. (Taken from ref. 18)

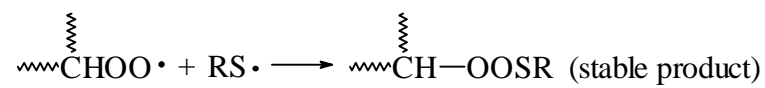
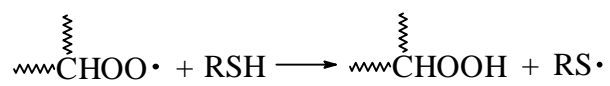
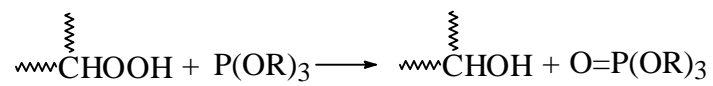


Figure 1.9. The mechanism of secondary additives. (Taken from ref. 17)

Several types of additives may be employed in a single polymer product. For example, both primary and secondary antioxidants, UV stabilizers, antiblocks, antistatic agents, blowing agents, and flame retardants may all be incorporated into low density polyethylene (LDPE) to maintain its performance.

In this research, we dealt with a primary antioxidant package that contained five different hindered phenols [i.e., BHT, BHEB, Isonox 129, Irganox 1076, and Irganox 1010 (**Table 1.4** and **Figure 1.7**)]. More specific physical properties of these antioxidants are tabulated in **Table 1.5**. In general, the molecular weight of the compound will be related to its temperature stability¹⁹. For instance, BHT has a molecular weight of 220 as compared with Irganox 1010 that has a molecular weight of 1178. Irganox 1010 would have a higher thermal stability than BHT. The relative volatilities of each are demonstrated in **Figure 1.10**¹⁸. This antioxidant additive package was incorporated into an LDPE product in order to avoid oxidative degradation.

¹⁹ Becker R.F., Burton L.P.J., Amos S.E. (1996) Polypropylene Handbook, ed. Moore E.P. Jr., Montell U.S.A., Inc.

Table 1.5. Physical properties of five target antioxidants.

Name	Melting point (°C)	Boiling point (°C)	Comment
BHT	69-70	265	-
BHEB	44-45	275	-
Isonox 129	162-164	-	-
Irganox 1076	49-52	-	No decomposition after eight hours at 185 °C in air
Irganox 1010	120	-	Stable at temperature in excess of 316 °C in air

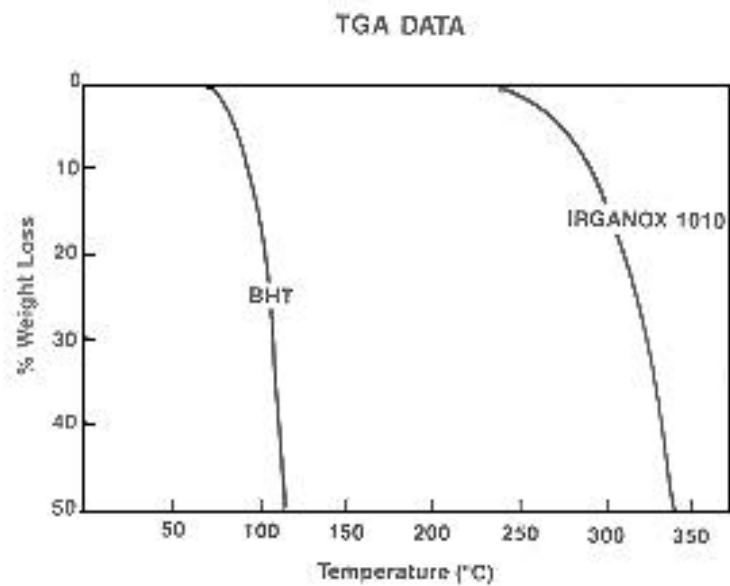


Figure 1.10. The relative volatilities of BHT and Irganox 1010. (Taken from ref. 18)

Thermogravimetric analyses (TGA) were conducted at a heating rate of 20°C per minute in an atmosphere of air.

Polymer Additive Analysis

As the purity and amount of additives incorporated into a polymer product affect the properties of the polymer, there is a need for reliable and rapid analytical methods to characterize the additives and to determine the amount of additive present.

Analysis of polymer additives can be complicated owing to their physical properties and the added problem of quantitative and reproducible extraction of the additives from the polymer matrix. Traditional liquid solvent/polymer extraction methods are time-consuming and uneconomical. They involve dissolution of the polymer in a hot solvent such as toluene²⁰ or decalin²¹, followed by precipitation of the high molecular weight fraction with an alcohol or by cooling. The optimal recoveries are usually low (<90%). Further separation is usually required prior to analysis. Analysis by GC is limited²² because of the problems in eluting non-volatile, high molecular weight additives. Aluminum-clad high temperature capillary GC columns have been used but such methods have been found to lack reproducibility²³. Owing to the relatively high molecular weight, reactivity, polarity, thermolability, and volatility of certain additives, LC has been the most commonly used chromatographic technique²⁴⁻²⁷. Unfortunately, many additives show little UV absorption. Because of the lack of a universal LC detector²⁸ and, in some cases poor resolution, LC has not proved ideal. Because of the low concentrations and complicated structures of additives, spectroscopic methods²⁹ and mass spectrometry (MS) have proved unsuccessful.

²⁰ British Standards 2782 (1965) BS Institution, London, Part 4, method 405D

²¹ Schabron J.F., Fenska L.E. (1980) *Anal. Chem.* **52**, 1411

²² DiPasquale G., Giambelli L., Sothenhni A., Pailla R. (1985) *J. High Resolut. Chromatogr. Chromatogr. Commun.* **8**, 618

²³ Cortes H.J., Bell B.M., Pfeiffer C.D., Graham J.D. (1989) *J. Microcol. Sep.* **1**, 278

²⁴ Dong M.W., DiCesare J.L. (1983) *Plastics Engineering* **2**, 25

²⁵ Bayloq D., Majcherczyk C., Pellerin F. (1985) *Ann. Pharm. Fr.* **43**, 329

²⁶ Howard J. (1971) *J. Chromatogr.* **55**, 15

²⁷ Hanely M.A., Dark W.A. (1980) *J. Chromatogr.* **18**, 665

²⁸ Schaborn J.F., Smith V.J., Ware J.L. (1982) *J. Liq. Chromatogr.* **5**, 613

²⁹ Freitag W., Fresenius Z. (1983) *Anal. Chem.* **316**, 495

Recent studies³⁰ have shown that SFE was at least as efficient as conventional liquid/solid extraction, and less time-consuming. SFC techniques have been demonstrated to be highly useful for determination of polymer additives³¹, particularly owing to the ability to use mass-sensitive FID.

Coupling SFE to SFC has become a most widely used two-dimensional technique in this field. Off-line SFE still dominates over on-line determination of additives, an important reason being the need for representative sample sizes. Small samples, however, allow a high linear velocity in the extractor, reducing the extraction time, and also diminish the build-up of extracted polymer material in restrictors and tubing. Thus, on-line SFE/SFC for determination of additives in polymers has a considerable growth potential.

Ashraf-Khorassani *et al.*^{13,32-33} investigated quantitative determination of a variety of polymer additives using the technique of SFE/cryogenic trapping/SFC. A polyethylene glycol (PEG) silica based packed column was used for SFC separation and FID was employed for detection. Extraction efficiencies of polymer additives from a number of different matrices were measured by varying the extraction conditions. Trapping efficiencies of the cryogenic accumulator were determined. Calibration curves of spiked pure LDPE pellets were determined. About 14 different polymer additives from different polymer matrices (e.g., LDPE, styrofoam, and propellant stabilizers) were quantified, the recoveries were greater than 92%, except for hexabromocyclododecan (HBCD) in styrofoam, which was 86%. Normal hydrocarbons have been determined by the hyphenated technique. It was found that the amount of material extracted and detected was directly proportional to the volume of sample placed in the extraction vessel. Peak areas with good relative standard deviation (%RSD<5.4, n=3) were reported.

Various polyethylene (PE) and polypropylene (PP) samples from several

³⁰ Hirata Y., Okamoto Y. (1989) *J. Microcol. Sep.* **1**, 46

³¹ Markides K.E., Lee M.L. (1988) The 1988 workshop on supercritical fluid chromatography, Park City, Utah.

³² Ashraf-Khorassani M., Boyer D.S., Levy J.M. (1991) *J. Chromatogr. Sci.* **29**, 517

³³ Ashraf-Khorassani M., Kumar M.L., Koebler D.J., Williams GP (1990) *J. Chromatogr. Sci.* **28**, 599

manufacturers were extracted using on-line SFE/SFC by Ryan *et al.*³⁴. Extraction efficiencies of greater than 92% were obtained for ten different additives including BHT and Irganox 1010. The recovery was relative to commercially available data. After extraction the additives were trapped on an accumulator column containing cyano-trapping material. Separation was achieved using a packed octyl column. Both FID and UV detection were used. Linear calibration curves for quantitation were constructed by extracting additive standards spiked onto quartz wool in the extraction vessel. Different extraction pressure and time were employed for the extractions of polymer samples while extraction temperature, accumulation temperature, and desorption temperature remained constant.

Cotton *et al.*³⁵ described the quantitative extraction and separation of additives and oligomers from PP and a number of other polymers. Fused silica capillary columns were used to perform the SFC separation at 120°C with pressure programming. Quantitative extraction of additives from PP was investigated at five different extraction pressures, at constant flow rate and temperature (i.e., 70°C). Below 50 atm, extraction was negligible; between 50-200 atm, Tinuvin 326 and 770 were extracted, along with small quantities of oligomers, although not in the ratio anticipated from their relative concentration in the material. High pressures led to the extraction of all the additives present. The relative peak area was calculated to compare with the relative composition. It was found that the integrated peak areas corresponded well with the actual concentration. The mass and the coefficient of variation (%RSD=8) for extraction and chromatography of the extracted cyclic trimer were determined, however, no quantitation method was reported.

The use of on-line SFE/SFC was described by Hirata *et al.*³⁶. A packed capillary column (fused silica tubing packed with ODS) was employed. PE film was extracted with

³⁴ Ryan T.W., Yocklovich S.G., Watkins J.C., Levy E.J. (1990) *J. Chromatogr.* **505**, 273

³⁵ Cotton N.J., Bartle K.D., Clifford A.A., Ashraf S., Moulder R., Dowle C.J. (1991) *J. High Resolut. Chromatogr.* **14**, 164

³⁶ Hirata Y., Nakata F., Horihata M. (1988) *J. High Resolut. Chromatogr. Chromatogr. Commun.* **11**, 81

supercritical CO₂ and the analytes were trapped on a 15 cm length uncoated fused silica tubing. By coupling a 5 cm section of this tubing to a packed capillary column and using direct injection, they were able to confirm that the extracts were efficiently trapped in the first 5 cm section, even at an extraction temperature of 65°C. The feasibility of extending the technique to quantitative studies was also demonstrated. However, no quantitative data were reported in this research.

In a further study by Daimon and Hirata³⁷, the use of uncoated and differently coated (film thickness) capillaries for concentrating extracted solutes was evaluated by comparing the recoveries of C₁₂ to C₂₀ alkanes at room temperature. The capillary with a 0.25 µm film was determined to be the most efficient trap. The effects of trapping temperature and extraction time on trapping efficiency were also studied using n-paraffin standards and polymer additive standards. They found that cooling the trapping tube during extraction would improve the trapping efficiencies. They also found that extraction efficiency of the additives from PP increased with increasing temperature. By varying extraction pressure and temperature, selective extraction was performed. However, no quantitative data were reported.

The variable character of the system's CO₂ gas flow rates over time due to deposition of material at the restrictor outlets caused detector calibrations and split ratios to change has been noticed by Baner *et al.*³⁸. The concentration of CO₂ in air at the FID and split restrictor outlets was measured in order to provide a basis for reproducible work with the on-line SFE/SFC system. The extraction conditions were optimized by extracting Biopol under the same pressure at different CO₂ gas flow rates. Triacetin (TA) standards with on-line SFE/SFC gave a recovery of 96.4% relative to the amount placed in the extraction vessel with a coefficient of variation of 19.7%. When extracting TA from Biopol polymer, 7.3% TA (w/w) with a coefficient of variation of 19% was obtained compared to the actual TA content of 6.8%. Commercial PP films were extracted to test

³⁷ Daimon H., Hirata Y. (1991) *Chromatographia* **32**, 549

³⁸ Baner L., Bucherl T., Ewender J., Franz R. (1992) *J. Supercritical Fluids* **5**, 213

the system. They also found 0.79% extractable material was in PP film with on-line SFE/SFC compared to 0.51% with Soxhlet extraction. It was found that 3.0 and 9.5% of the total chromatogram area units for Irganox 1076 and 1010 respectively were obtained compared to 2.9 and 20.3% with Soxhlet extraction. The coefficient of variation of Irganox 1076 and 1010 were 25 and 15% respectively.

On-line SFE/SFC has also been described by MacKay and Smith³⁹ using cryogenic trapping to concentrate analytes at the top of the analytical column prior to chromatography. Four chlorinated organophosphate flame retardants present in polyurethane foams were analyzed. Both FID and MS were employed to confirm the identity of the retardants. An external calibration with standards injected into SFC was prepared for on-line quantitative extraction. Good recoveries were obtained for all retardants except Amgard V6 from “Safegard” due to its low solubility in supercritical CO₂.

Oudsema and Poole⁴⁰ reported on-line SFE/SFC with formic acid modified CO₂ to determine an organotin stabilizer in a rigid polyvinyl chloride (PVC). A cyanopropyl packed column was used for separation. A solution of formic acid was loaded by syringe into the pump cylinder head to achieve 0.3% (v/v). A cryogenic stainless steel precolumn was used as a trap. The influence of temperature, pressure, and time on the extraction of the dimethyltin additive in a sample was investigated. Raising the temperature to 90°C from 60°C resulted in a fast extraction. At pressures greater than 150 atm the recovery of the analyte was unaffected by increasing pressure at 90°C, which indicated that at the effective fluid density, the solubility was sufficiently high that the analytes reaching the surface of the polymer particles were rapidly transported to the interface for cryotrapping. A small reduction in recovery was observed for extraction times longer than 60 min at 90°C and 175 atm. Trapping temperatures greater than 30°C were not adequate for quantitative trapping. The concentration of the additive was determined to be 1.45% with

³⁹ MacKay G.A., Smith R.M. (1993) *Analyst* **118**, 741

⁴⁰ Oudsema J.W. Poole C.F. (1993) *J. High Resolut. Chromatogr.* **16**,198

an RSD of 2.9% (n=6). However, no actual concentration was reported.

In order to obtain high extraction efficiency, polymer samples are usually ground, shaved or filed to increase the surface area. It is time-consuming and, in many cases, may result in thermal degradation of some analytes through the heat produced. In order to avoid these problems, MacKay and Smith⁴¹ demonstrated a method for the quantitative analysis of Tinuvin P from unplasticized PVC film using an internal standard with an on-line SFE/SFC system. The internal standard which would be extracted at the same or similar rate to the analyte, could be incorporated into the polymer matrix, then the ratio in an incomplete extraction could be used to determine the initial concentration. Tinuvin 326 was selected as an internal standard because it has similar properties as the target analyte, namely, similar solubility, diffusion rate, similar interaction with the polymer matrix and similar FID response. The response ratio of the analyte to the internal standard was determined by a solution containing the same concentration of each compound with SFC. A single point calibration was employed. The internal standard was then incorporated into the bulk plastic sheet, therefore, complete extraction was not necessary to gain quantitative information with good reproducibility (standard deviation = 4.3%) in a short extraction time. However, no recovery was reported. In addition, incorporation of the internal standard introduced additional sample preparation stages such as dissolving the polymer in solvent and evaporating the solvent, which might detract from the usefulness of this method of assay.

In our study, efforts have been made to quantify different additives in LDPE using on-line SFE/SFC. In the first part of the study, spiking experiments (on sand) were performed to investigate the influence of different traps and trapping temperature, injection temperature, extraction pressure, extraction temperature, fluid flow rate, and extraction time upon trapping and extraction efficiencies. The second part of our study involved quantitation of the additives from LDPE using previously determined conditions.

⁴¹ MacKay G.A. Smith R.M. (1995) *J. High Resolut. Chromatogr.* **18**, 607

Off-line SFE/HPLC and off-line ESE/HPLC was conducted in the third part to compare with the on-line SFE/SFC technique.

Chapter 2

Quantitative Analysis of Additives from Low Density Polyethylene

Introduction

On-line SFE/SFC with pure CO₂ was employed to analyze and quantitate different additives in LDPE. The influences of trap packings, trapping temperature, extraction pressure and temperature, extraction time, fluid flow rate, and thermal desorption temperature were investigated. Optimized conditions were employed to quantitatively extract the additives from LDPE. Off-line SFE/HPLC and off-line Enhanced Solvent Extraction (ESE)/HPLC were conducted on the same sample. The results obtained from on-line SFE/SFC were compared to those from off-line SFE/HPLC and off-line ESE/HPLC. The main objective of this study was to develop a quantitation method using on-line SFE/SFC to analyze polymer additives from LDPE product.

Experimental

Material

The following polymer additives were analyzed: BHT, BHEB, Isonox 129, Irganox 1076, and Irganox 1010. The chemical name, chemical structure, and physical properties were given in **Table 1.4**, **Figure 1.7**, and **Table 1.5**.

Additive standard mixtures at various concentrations were prepared using methylene chloride (CH₂Cl₂) as solvent. An additive standard mixture with a concentration of 5000 ppm for each additive standard was first made and successively diluted to encompass additive concentrations of 100-5000 ppm.

Additive standards were provided by Quantum Chemical Corporation (Cincinnati, OH), as well as the LDPE sample (20 mesh), originally containing approximately 1000 ppm of each additive. The glass transition temperature of LDPE is well below ambient temperature, and the melting point is 106-115°C.

Methods

On-line SFE/SFC

An Isco-Suprex (Lincoln, NE) MPS/225 SFE/SFC consisting of a supercritical fluid extractor, cryogenic collection trap (CC), and supercritical fluid chromatograph was utilized to perform on-line extraction, collection, and separation. The SFE/CC/SFC system consisted of a 0.16 mL stainless steel extraction vessel and a cryogenic collection tube measuring 30 × 1.0 mm i.d., which had the capabilities of rapid cooling to –50°C (200°C/min) and ballistic heating up to 200°C (250°C/min). A Deltabond™ cyano column, 100×1.0 mm i.d., 5 μm particle size, was used to provide the SFC separation. Pure CO₂ with helium headspace was used as the mobile phase.

In addition to the above, the SFE/CC/SFC assembly was composed of three electronically actuated valves (ten-port, two-position valve; five-port, four-position selector valve; and four-port, two-position injector valve). Schematic diagrams of the extraction, cryogenic collection trap, valving, and chromatographic column in the extraction/collection mode and in the injection/separation mode are shown in **Figure 2.1**. In the extraction/collection position (**Figure 2.1A**), CO₂ from the syringe pump enters the tee. Tubing from one outlet of the tee leads CO₂ to the injector valve for use only in conventional SFC applications. Tubing from the other outlet of the tee goes through the five-port, four-position selector valve to the extraction vessel. The extracted components carried by CO₂ pass through a linear fused silica restrictor to the ten-port, two-position valve, and then into the cryogenic collection trap, which is cooled as low as –50°C with industrial-grade CO₂. All of the extracted materials are then collected in the cryogenic trap, while the expanded CO₂ gas from the cryogenic collection trap is vented through the ten-port valve into the atmosphere.

After extraction is completed, the system pump pressure is reduced from the extraction pressure (e.g., 450 atm) to the starting pressure (e.g., 100 atm) for

chromatography. During this re-equilibration period, the sample remains in the cryogenic collection trap. Upon reaching equilibrium the ten-port and five-port selector valves are switched simultaneously to the injection/separation mode (**Figure 2.1B**). In this configuration, CO₂ passes through the tee, the selector valve, and the ten-port valve into the cryogenic trap, which is then ballistically heated to desorption temperature (e.g., 180°C). After backflushing, CO₂ carries the extracted components from the trap back to the ten-port valve and into the packed chromatographic column. The sample passes through a tapered fused silica restrictor before it reaches the FID, while depressurized CO₂ is vented out from FID to the atmosphere.

An additive standard mixture was spiked onto about 0.3 g Ottawa sand (Fisher Scientific, Fairlawn, NJ) contained in the 0.16 mL extraction vessel to investigate the effects of various parameters (e.g., trap and trapping temperature, extraction pressure, temperature, time, fluid flow rate, etc.) upon extraction efficiency and collection efficiency. Experiments were performed in triplicate. The SFC conditions were previously developed in our lab to separate the five additives efficiently. **Figure 2.2** shows one on-line SFE/SFC chromatogram of sand spiked with the additive standards.

The following extraction and chromatography conditions were employed for on-line SFE/SFC:

SFE conditions:

Extraction fluid:	100% CO ₂
Pressure:	350, 450 atm
Oven temperature:	80, 100°C
Time:	10, 20, 30, 40, 50 min
Cryogenic trapping temperature:	-50, -40, -25, -5, 5 °C
Flow rate:	0.65, 2.0 mL/min

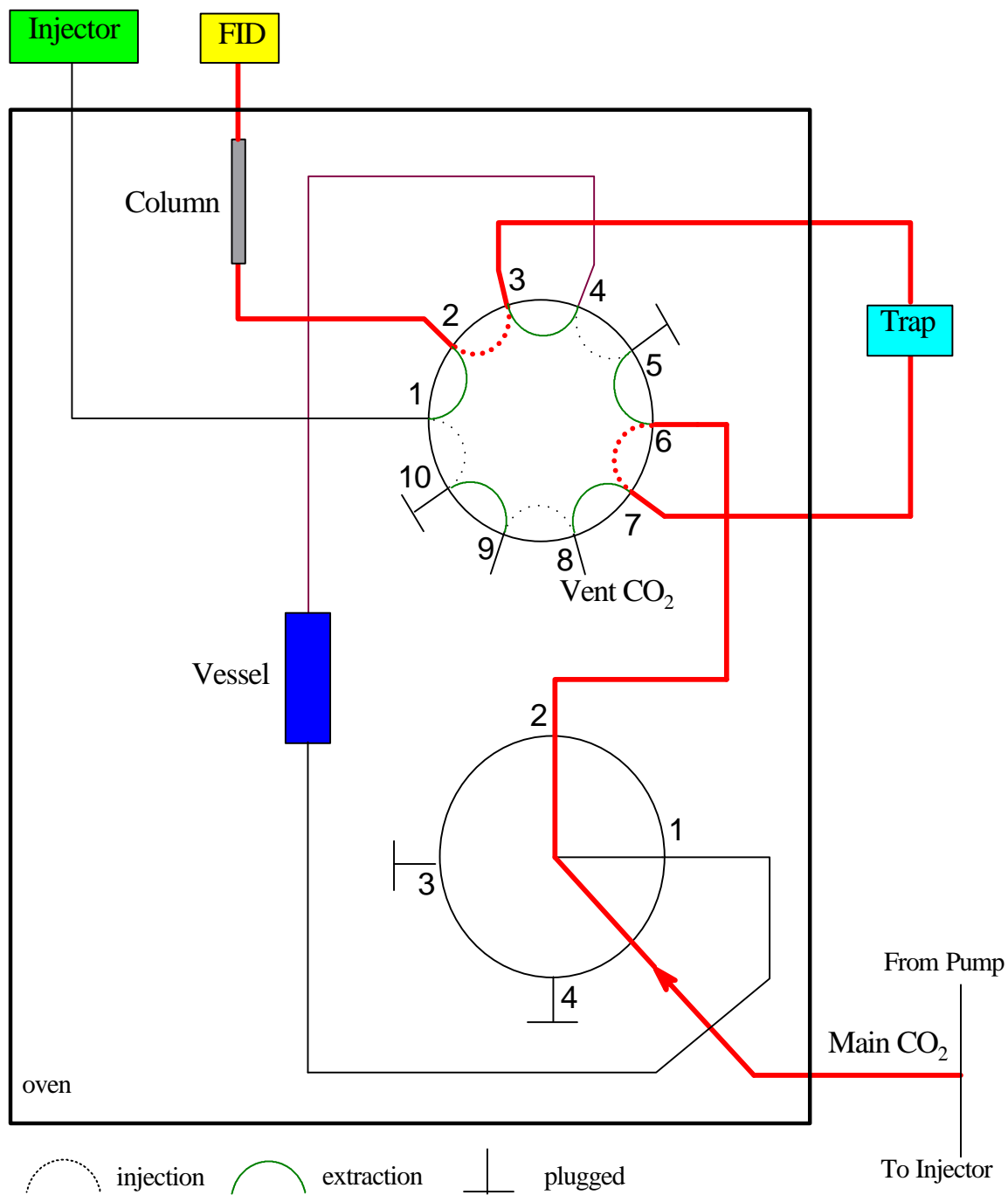


Figure 2.1B. Schematic diagram of on-line SFE/SFC, injection/separation mode.

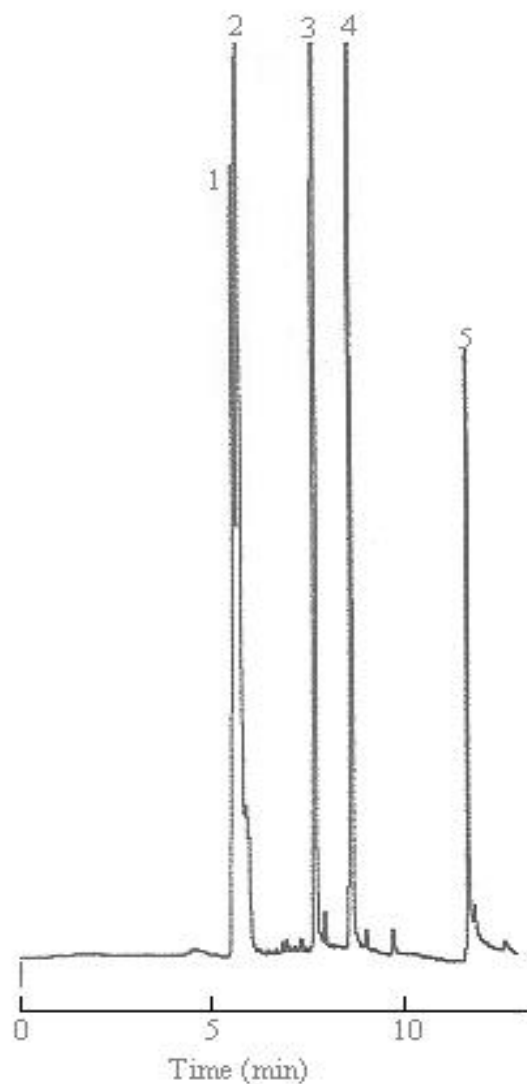


Figure 2.2. On-line SFE/SFC chromatogram of spiked sand with additive standards.

1. BHT, 2. BHEB, 3. Isonox 129, 4. Irganox 1076, 5. Irganox 1010.

SFE conditions: 450 atm, 100°C, 30 min, trapping at -40°C.

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100×1.0 mm, 5 μm d_p, FID detection at 350°C.

SFC conditions:

Pressure programming:	100 atm for 3 min 100-330 atm in 7 min 330-450 atm in 1.5 min 450 atm for 5 min
Oven temperature:	100 °C
Desorption temperature:	100, 180 °C
Column:	100×1.0 mm i.d., 5 µm d _p Deltabond™ cyano

Spiked sand was also employed to prepare calibration curves for quantitation. The spiked sand was air dried for about one minute. The LDPE sample was subjected to on-line SFE/SFC to obtain qualitative and quantitative results of the target additives. All experiments were performed in triplicate. Identification was achieved by comparing the retention time of each additive standard.

Off-line SFE/HPLC

An Isco-Suprex AP44 automated extraction system equipped with an automatic variable restrictor and Accutrap™ collection system was used with the following conditions previously developed by Quantum Chemical Corporation:

Extraction fluid:	100% CO ₂
Oven temperature:	100 °C
Pressure:	450 atm
Restrictor temperature:	75 °C
Vessel size:	5 mL
Liquid flow rate:	1.5 mL/min
Dynamic extraction time:	30 min
Solid phase trap:	octadecyl silica (ODS) at 0 °C
Trap desorb temperature:	25 °C

Trap rinse: 5 mL 50/50 ethylacetate/acetonitrile

The extraction vessel was filled to approximately 80% of the volume with Ottawa sand. For the LDPE polymer sample, 500 mg was added onto the sand. A small dead volume was necessary due to expansion of the polymer during extraction.

For the HPLC portion of the analyses, a Hewlett Packard (Wilmington, DE) series 1050 HPLC was used with the following parameters:

Column: 150×3.9 mm, 5 μ m d_p , C₁₈

Column temperature: 50°C

UV detector: 200 nm

Mobile phase: Gradient from 75/25 (v/v) CH₃CN/H₂O to 100% CH₃CN in 5 min, hold 100% CH₃CN for 14 min, return to 75/25 (v/v) CH₃CN/H₂O at 19.01 min

Flow rate: 1.5 mL/min

Sample loop: 10 μ L

All solvents were HPLC grade and were obtained from Fisher Scientific (Fair Lawn, NJ).

Quantitation was accomplished by using an external calibration. The LDPE sample was also subjected to off-line SFE/HPLC. All experiments were performed in triplicate. However, the sample size used in off-line SFE/HPLC was much larger (500 mg vs. 2.0 mg) than that in on-line SFE/SFC.

Off-line ESE/HPLC

An Isco SFX 220 SFE system (**Figure 2.3**) was modified to conduct ESE. The system consisted of two syringe pumps and an oven. A 10 mL extraction vessel was placed in the oven. A static extraction was performed at 200 atm and 100°C for 30 min with 10 mL 50/50 ethylacetate/CH₃CN. The extract along with the solvent was collected in a vial, and 10 mL CO₂ was used to flush the solvent out of the extraction vessel. The

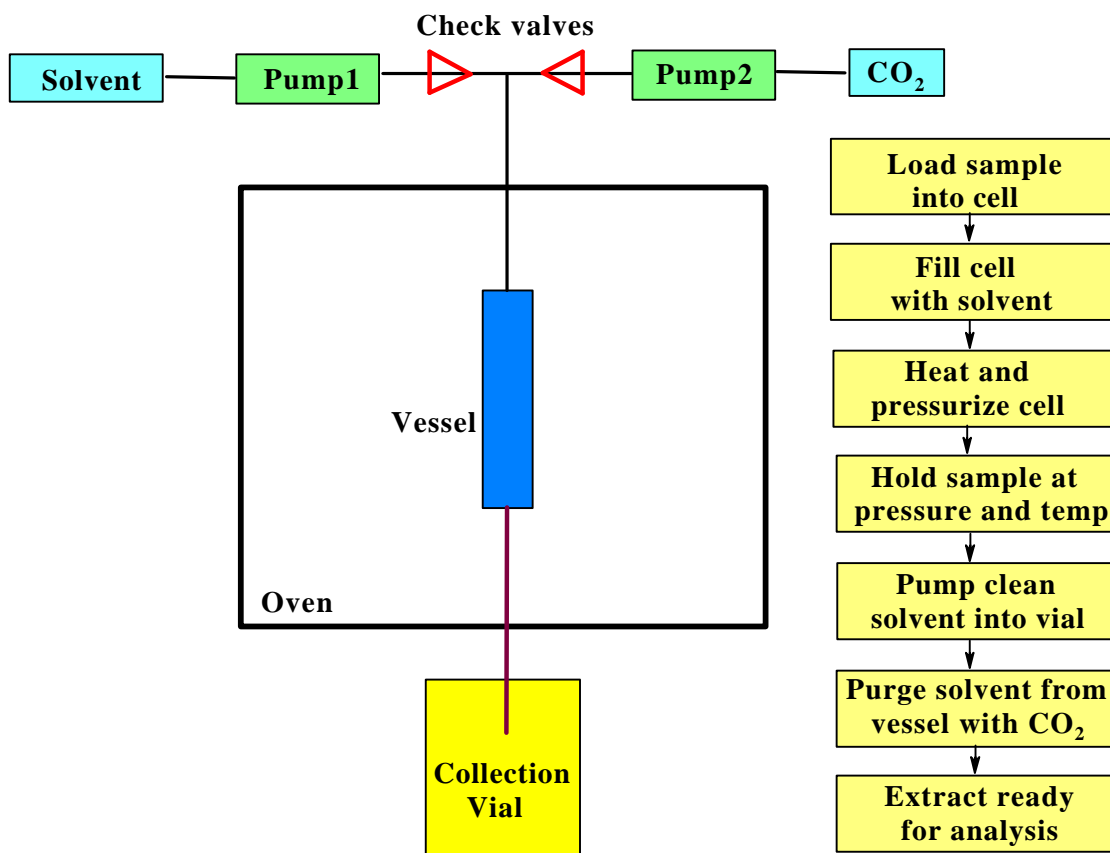


Figure 2.3. Schematic diagram of ESE system used in our study.

tube after the extraction vessel was easily clogged with the messy extracts. Post-extraction clean-up was therefore necessary. The sample used for ESE was 500 mg.

Data Analysis

F-test and t-test were employed to statistically analyze the data obtained from the experiments. An F-test is a method for comparing two population variances, i.e., the random errors (precision) of two sets of data. The F-test considers the ratio of the two sample variances, i.e., the ratio of the squares of the standard deviations. The quantity calculated (F) is given by:

$$F_{calc} = \frac{S_1^2}{S_2^2} \quad (\text{Equation 1})$$

Where S_1 and S_2 are the standard deviations of each data set. S_1 and S_2 are allocated in the equation so that F_{calc} is always ≥ 1 . If the calculated value F exceeds a certain critical F value, which depends on the size of both the samples, the significance level and the type of test performed, then the precisions of two data sets are significantly different.

Two-sample assuming unequal variances t-test is a method to determine whether two sample means are equal. This form of t-test assumes that the variances of both ranges of data are unequal and is referred to as a heteroscedastic. Two-sample assuming equal variances t-test is a method to determine whether two-sample means are equal when the variances of both ranges of data are equal, which is referred to as a homoscedastic. A F-test was always performed first to determine whether there was significant difference in precision between two data sets. Either type of t-test was then performed to determine whether equivalent data were obtained at different conditions. Paired two-sample for means is a method to determine whether a sample's means were distinct, and does not assume that the variances of both populations from which the data sets are drawn are equal. This form of t-test was performed to determine if different methods provided

equivalent results when all analytes were concerned. The following equation is used to calculate t value:

$$t_{calc} = \frac{|\bar{x}_1 - \bar{x}_2|}{S_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \quad (\text{Equation 2})$$

Where x_1 and x_2 are the average values of each of the two data sets, S_p is the pooled standard deviation, and n_1 and n_2 are the number of data points in each set. If the calculated t value exceeds critical t value, the means of two data sets are significantly different.

The confidence level used for F-test and t-test in this work was 95%⁴².

Figure 2.4 shows an example of Microsoft Excel statistics worksheet to compare the percent recoveries of Isonox 129 obtained from on-line SFE/SFC and off-line SFE/HPLC. The F-test was performed to obtain the calculated F value, 817, which exceeds the critical F value, 19, therefore, the precision obtained from off-line SFE/HPLC for Isonox 129 was much better than that from on-line SFE/SFC. Next the t-test for two-sample assuming unequal variances was employed to determine whether the means were significantly different. The calculated t value, 1.294, is smaller than the critical t value, which means equivalent percent recoveries were obtained from two methods for Isonox 129.

⁴² Miller J.C., Miller J.N. (1988) Statistics for Analytical Chemistry John Wiley & Sons, Inc., New York

%Recovery of Isonox 129			
on-line SFE/SFC	63	80	96
off-line SFE/HPLC	67	67	68

F-Test Two-Sample for Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	79.7	67.3
Variance	272.3	0.3
Observations	3	3
df	2	2
F	817.000	
P(F<=f) one-tail	0.001	
F Critical one-tail	19.000	

t-Test: Two-Sample Assuming Unequal Variances

	<i>Variable 1</i>	<i>Variable 2</i>
Mean	79.7	67.3
Variance	272.3	0.3
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	1.294	
P(T<=t) one-tail	0.163	
t Critical one-tail	2.920	
P(T<=t) two-tail	0.325	
t Critical two-tail	4.303	

Figure 2.4. An example of Excel statistics worksheet.

Results and Discussion

On-line SFE/SFC

CO₂ impurities

One important consideration with an on-line SFE/SFC system is the impurities in CO₂. In a standard SFC, the low concentration level of hydrocarbon impurities in the mobile phase cannot be detected by FID, When a UV detector is employed, hydrocarbons cannot be detected either, due to the lack of a chromophore. Interestingly, the high molecular weight hydrocarbon (i.e., C₁₈-C₃₀)^{33,43} impurities in SFE/SFC grade CO₂ are accumulated in the cryogenic trap.

To study this issue, on-line SFE/SFC/FID was conducted with three different tanks of CO₂. A total of 15 g of CO₂ was passed through the cryogenic collection system from each cylinder, and the impurities in CO₂ were collected at -25°C for 30 min. After collection of the impurities, a SFC chromatogram was obtained for each tank. It was observed (**Figure 2.5**) that the level of impurities varied from tank to tank. It is important to note that if the level of impurities in CO₂ is too high it may interfere with the peaks in the sample chromatogram.

In order to eliminate the interferences from the impurities, a stainless steel column measuring 25×2.5 cm i.d. was used as a filter before the CO₂ entered the on-line SFE/SFC system. It contained ¾ activated carbon (4-12 mesh) and ¼ adsorption alumina (80-200 mesh), which was suggested by Air Products & Chemicals Inc., to adsorb the hydrocarbon impurities in CO₂ tank. **Figure 2.5D** shows a chromatogram obtained from on-line SFE/SFC/FID of 15 g of CO₂ with the filter. The same conditions as above were employed, and no obvious impurity peaks were observed.

Memory Effect

Another issue with the on-line SFE/SFC system is the so-called “memory effect”, which is defined as the failure to remove of extracted analytes even after several purges of

⁴³ Engelhardt H., Zapp J., Kolla P. (1991) *Chromatographia* **32**, 527

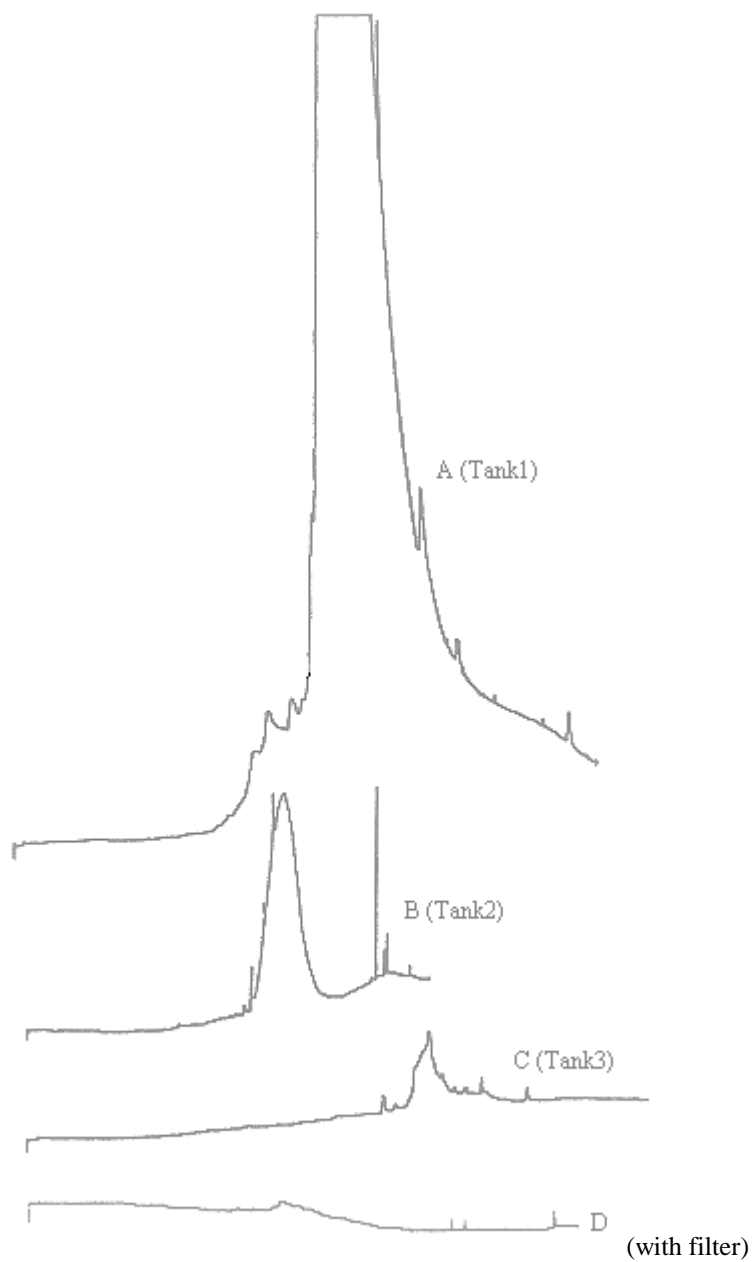


Figure 2.5. Chromatograms of impurities in CO₂ tanks, integration at same attenuation 3.

SFE conditions: 450 atm, 100°C, 30 min, trapping at -25°C.

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100×1.0 mm, 5 μm d_p, FID detection at 350°C.

the system with extraction fluid. The deleterious effect may not present itself immediately, but rather when experimental conditions are changed. The undesired result may show up, for example, as an unexplained analyte peak in a further chromatogram due to removal of the old analyte from the various places. For the system conducted with the same analytes, bad quantitation reproducibility may occur.

Previous study by Hume-Kirschner *et al.*⁴⁴ showed that cleaning the trapping system with liquid CH₂Cl₂ in a modified on-line SFE/SFC could eliminate this “memory effect”. However, the simple test performed in that study to demonstrate the effect of cleaning was not of sufficient extraction time (5 min) for the trace residues to be enriched enough to be detected. In our study, at least 30 min extraction time was employed; therefore, the test extraction time should be no shorter than 30 min.

In our work, the dried spiked sand was extracted at 450 atm and 100°C for 30 min, and chromatography was conducted with pressure programming. Following this SFE/SFC performance, the vessel was removed and replaced with a zero dead volume union to make sure that no interferences from possible residue in the vessel was seen. Then another similar SFE/SFC was performed. **Figure 2.6** shows the chromatograms of the extractions of the “blank” system for 1 min and 30 min. As can be seen, the “memory effect” was exhibited when the 30 min extraction was conducted.

In order to eliminate this problem, several efforts were made, including changing the pre-trap restrictor, washing the extraction lines with CH₂Cl₂, and sonicating the rotor of the ten-port valve in CH₂Cl₂. All cleaning solvent was collected, concentrated, and injected into the SFC, but no analyte peaks were observed. After drying all the parts, with the union in place of the vessel, a 30 min extraction was performed followed by chromatography. Small quantities of residues were still evident. **Figure 2.7** shows the chromatograms after each cleaning step.

This process indicated that the residual (samples) could deposit unpredictably in some “dead space” (e.g., spaces between the restrictor tip and fitting, etc.) of the system,

⁴⁴ Hume-Kirschner C., Jordan S.L., Taylor L.T. (1993) Unpublished Results



Figure 2.6. Chromatograms of memory effect.

A. 1 min extraction, B. 30 min extraction.

SFE conditions: 450 atm, 100°C, 30 min, trapping at -25°C.

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100 × 1.0 mm, 5 μm d_p, FID at 350°C.

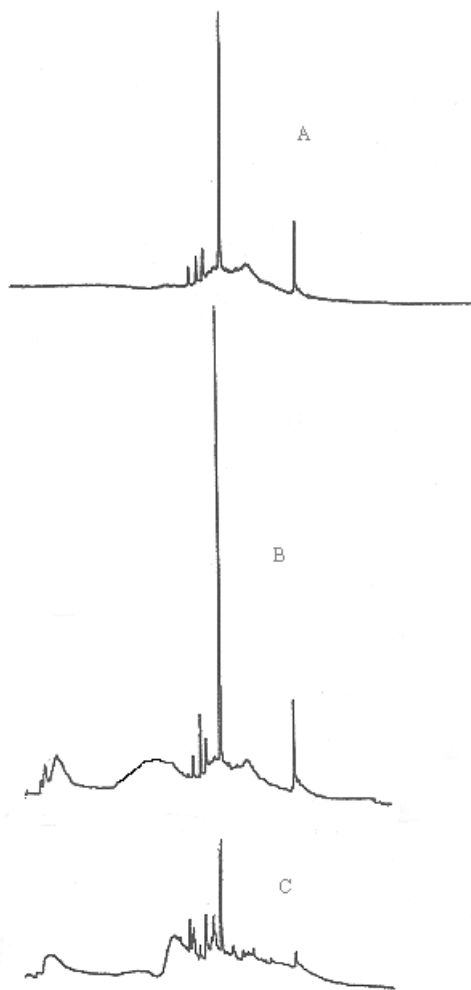


Figure 2.7. SFE/SFC after various cleaning steps.

A. Changing pre-trap restrictor, B. Flushing trapping line with CH_2Cl_2 , C. Sonicating the valve

SFE conditions: 450 atm, 100°C , 30 min, trapping at -25°C .

SFC/FID conditions: desorption at 180°C , pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C , Deltabond™ cyano, 100×1.0 mm, $5 \mu\text{m } d_p$, FID at 350°C .

which are very difficult to reach by regular flushing with CO₂ or solvent. However, the “memory effect” may not affect the results significantly when the sample concentration is sufficiently high.

Consideration of cryogenic trapping

Generally, the success of on-line SFE/SFC greatly depends on the trapping techniques to recover the extracted analytes from the expanded gas flow after depressurization, particularly when the analytes are volatile. The primary objective of this part of the work was to evaluate the efficiency of an empty cryogenic trap and a cryogenic trap filled with glass wool.

An ODS solid phase trap works great in off-line SFE systems. It seemed logical at first that ODS particles could probably be packed into the empty cryogenic trap of the on-line SFE/SFC system to improve collection efficiency. However, backpressure was found to suck the small particles into the valve and column and ruin them when the system is switched from SFE mode to SFC mode. In addition, some polymer additives are difficult to desorb from ODS with pure CO₂ when SFC is performed³⁷. Organic solvents such as CH₂Cl₂, CH₃CN, and CH₃OH were used in off-line SFE to rinse the analytes out of the ODS trap.

The efficiency of two cryogenic traps was examined using the additive standard mixture. Ten µL of the standard solution was loaded in the extraction vessel filled with sand. The solvent was allowed to evaporate for several minutes. Extraction was performed at 450 atm and 100°C. The trapping temperature was -40°C. The results shown in **Figure 2.8** clearly indicate that the trapping efficiency was improved significantly with glass wool filled in trap.

As mentioned above, the situation can be more serious when the analytes are relatively volatile. BHT is the one of the most volatile species among all the polymer additives¹⁸. **Figure 2.8** also shows the reproducibility (%RSD) obtained using the two cryogenic traps. Obviously, the empty cryogenic trap could not quantitatively collect

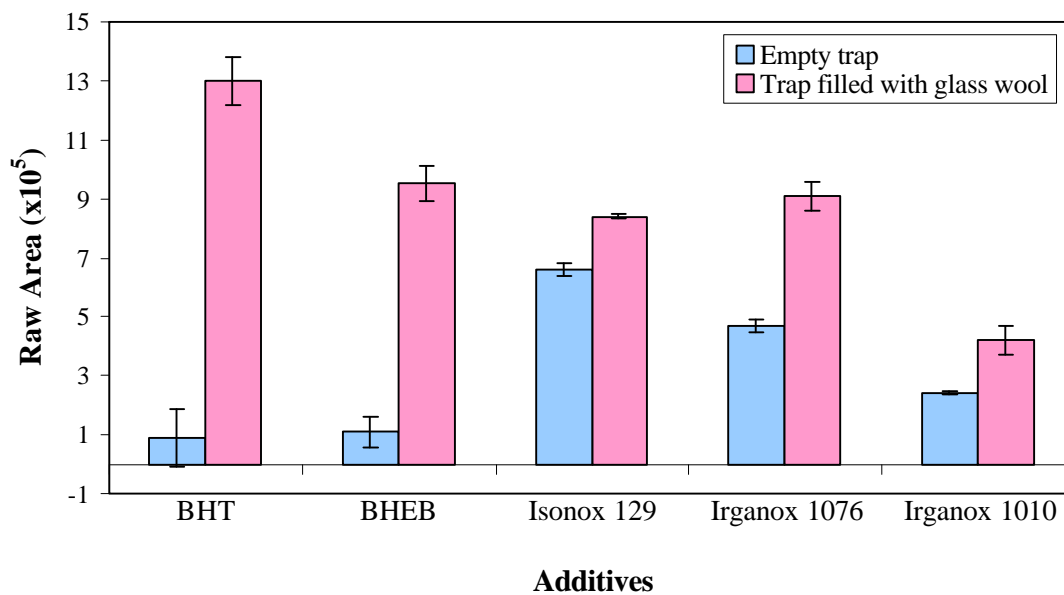


Figure 2.8. Comparison of different trapping protocols (Spiked sand).

SFE conditions: 100% CO₂, 450 atm, 100°C, 30 min, trapping at -40°C,
 SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3
 min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C,
 Deltabond™ cyano 100 × 1.0 mm i.d., 5 μm d_p, FID at 350°C.

volatile analytes such as BHT and BHEB. Therefore, the cryogenic trap filled with glass wool was employed in the following study.

The effect of trapping temperature on trapping efficiency was next investigated by spiking 10 μL of the 500 ppm additive standard mixture onto the sand. Extraction was performed at 450 atm and 100°C. The extracts were collected at different trapping temperatures (i.e., -50, -40, -25, -5, 5°C). After 30 min extraction/collection, the SFE/SFC valves were switched to the injection mode and collected materials were backflushed at 180°C to the separation column.

As can be seen in **Figure 2.9**, the effect of trapping temperature on the collection of relatively volatile species such as BHT and BHEB was profound. At trapping temperatures higher than -40°C, dramatic decreases in peak area were observed due to their relatively high volatilities. The analytes easily vented out of the trap with the expanded CO₂ at the higher temperatures. On the other hand, no significant difference was found for the collection of Isonox 129, Irganox 1076, and Irganox 1010 at different trap temperatures due to their stabilities at relatively high temperature. Therefore, the cryogenic trap was maintained at -40°C to ensure adequate trapping of this additive package.

The heating of the cryogenic collection trap prior to SFC was also investigated. In order to show the effect of heating at different temperatures on the efficiency of removing the extracts from the trap to the SFC column, the spiked sand was extracted and collected at -40°C. After 30 min of collection, the system was switched to the SFC injection mode while the cryogenic trap was ballistically heated to 100°C. A second SFC injection was performed with the thermal desorption temperature at 180°C without any additional extraction. The chromatograms are shown in **Figure 2.10**. By comparing the peak areas, we found even for volatile analytes such as BHT and BHEB, 2-3% of the extracted amount was left in the trap after the first injection at 100°C. The unremoved amounts after the first injection of Isonox 129, Irganox 1076, and Irganox 1010 were 21%, 11%, and 9%, respectively.

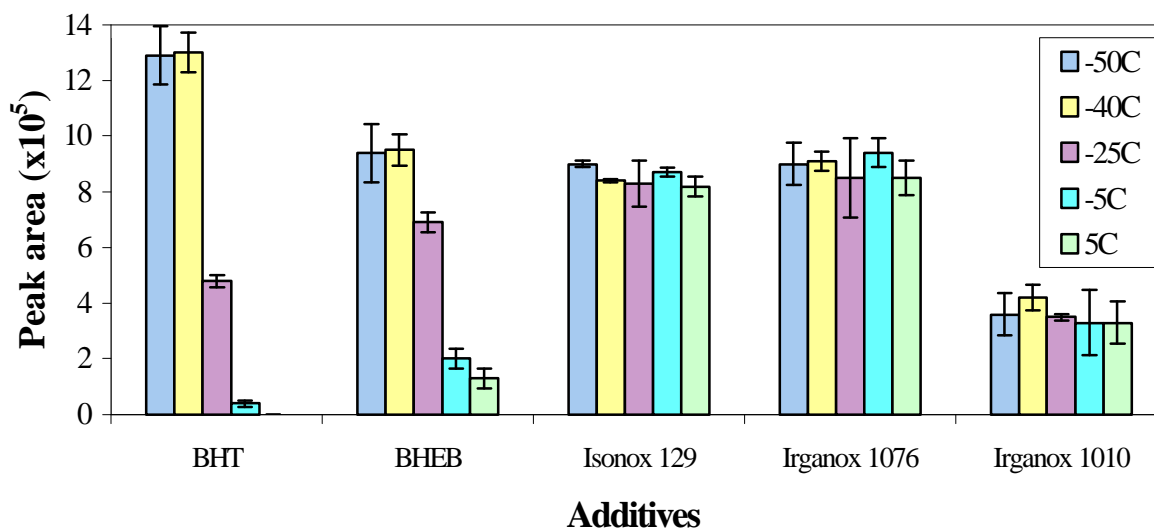


Figure 2.9. Effect of different trap temperatures on collection efficiency (Spiked sand, glass wool trap).

SFE conditions: 450 atm, 100°C, 30 min, flow rate: 0.65 mL/min.

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100 × 1.0 mm, 5 μm d_p FID at 350°C.

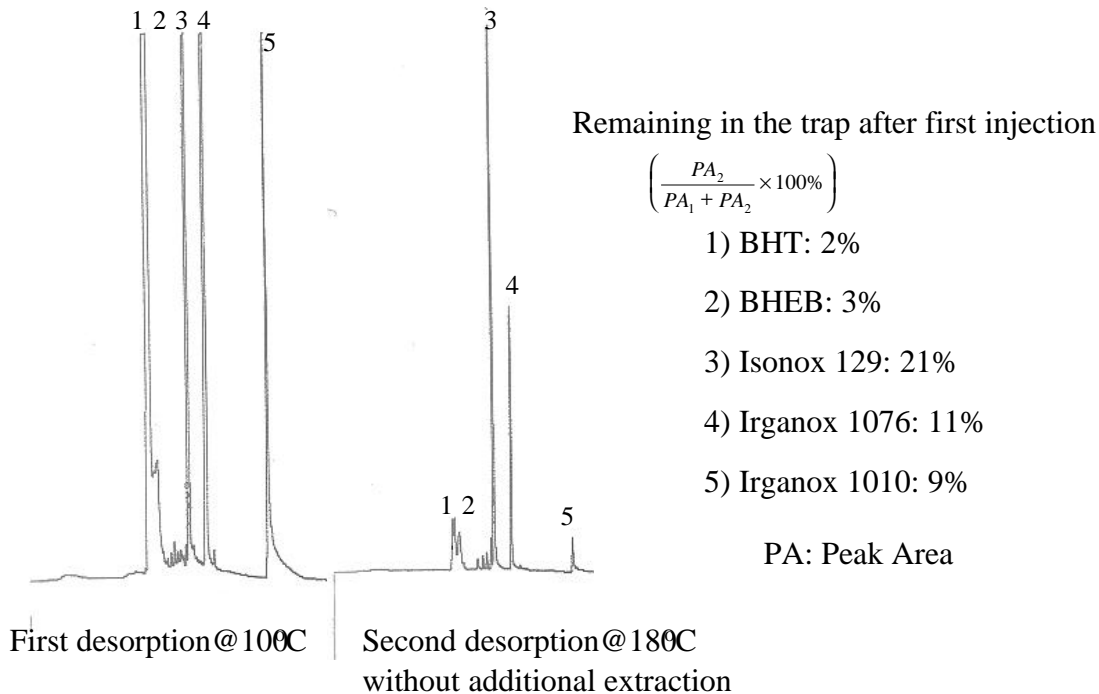


Figure 2.10. On-line SFE/SFC chromatogram of sand spiked with additive standards at different desorption temperatures.

A. 100°C, B. 180°C.

1. BHT, 2. BHEB, 3. Isonox 129, 4. Irganox 1076, 5. Irganox 1010.

SFE conditions: 450 atm, 100°C, 30 min, trapping at -40°C.

SFC/FID conditions: pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100×1.0 mm, 5 μm d_p, FID detection at 350°C.

When the injection was first performed at 180°C, a following second run showed that no significant amount of the analytes was left in the trap.

This result suggested that although lower SFE temperatures resulted in higher solvating power, the removal of the analytes from the trap area to the SFC column was dominated by not only the solubility in the fluid but also the higher temperature.

Optimization of Extraction Parameters

The main factors affecting the efficiency of extraction of the additives from polymers are the solubility of the additives in the fluid and the rate of mass transfer of the additives out of the polymer matrix^{35,37,45,46}. In the absence of specific additive-polymer matrix interaction (e.g., sand instead of polymer matrix), the extraction will primarily be controlled by pressure, temperature, and time, as well as fluid flow rate.

In our study, the effects of extraction pressure, temperature, time, and fluid flow rate were investigated by spiking the additive standards onto the sand using the optimized trap conditions. Results from the optimization study are summarized in **Figure 2.11-2.14**.

As seen in **Figure 2.11**, increasing the extraction pressure from 350 to 450 atm at constant extraction temperature leads to a statistical increase in the extraction of Irganox 1076 and Irganox 1010, while no significant variation in the extraction yields of the other three additives was observed. These results suggested that at 350 atm and 100°C, the density, and hence the solvating power of CO₂, was sufficiently high for the extraction of low molecular weight analytes such as BHT, BHEB, and Isonox 129. Further increase of the pressure didn't result in a further increase of the extraction yield. Apparently the effect of pressure on the extraction of high molecular weight species (i.e., Irganox 1076 and Irganox 1010) was more pronounced than that observed for low molecular weight analytes. It appeared that at 350 atm and constant temperature the solubilities of Irganox 1076 and Irganox 1010 in CO₂ were not sufficiently high. Increasing the pressure from

⁴⁵ Bartle K.D., Boddington T., Clifford A.A., Hawthorne S.B. (1992) *J. Supercritical Fluids* **5**, 207

⁴⁶ Bartle K.D., Boddington T., Clifford A.A., Cotton N.J. (1991) *Anal. Chem.* **63**, 2371

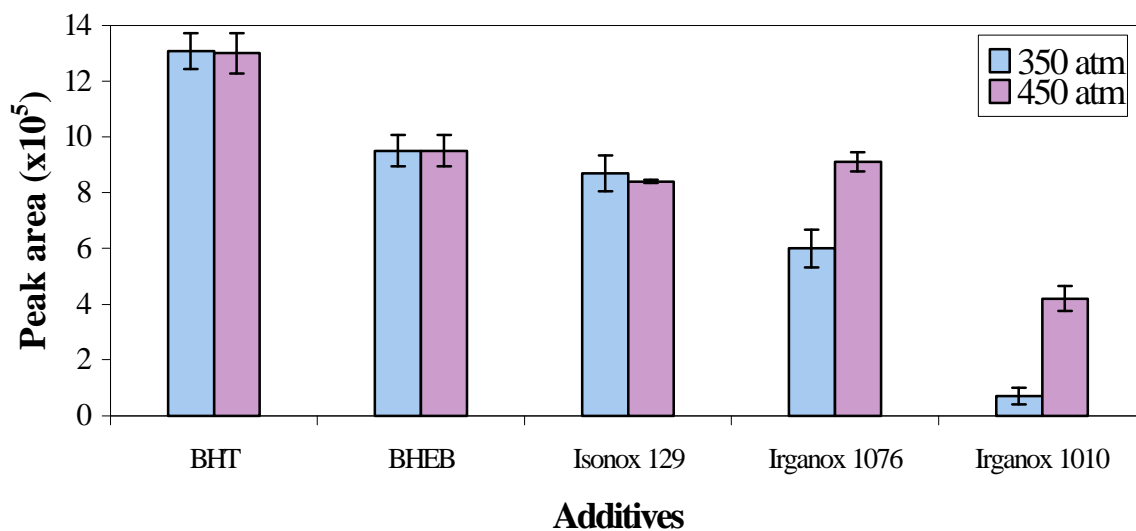


Figure 2.11. Effect of extraction pressure on extraction efficiency (Spiked sand).
 SFE conditions: 100°C, 30 min, trapping at -40°C, flow rate: 0.65 mL/min.
 SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100 × 1.0 mm, 5 μm d_p, FID at 350°C.

350 to 450 atm (density from 0.72 to 0.80 g/mL) caused the extraction rates to increase significantly. For BHT, BHEB, and Isonox 129, above a certain pressure value at constant temperature, it seemed that the solubility of the components in the SF is no longer the rate-limiting parameter for extraction, whereas for Irganox 1076 and Irganox 1010, the solubility may always be the rate-limiting parameter for the extraction even at quite high pressure due to their relatively poor solubility in CO₂ because of their high molecular weight⁴⁷.

The effects of temperature at constant pressure are even more complicated than the effect of pressure at constant temperature. Increasing temperature decreases solute-fluid interaction, which results in a decreased solvating power. Whereas at the same time it also decreases solute-solute interaction, which results in an increased solubility. Therefore two competing factors affect the extraction of the solute in the SF.

One important point must be kept in mind is special consideration for extraction temperature when real polymer samples are dealt. The glass transition temperature (T_g) and melt temperature (T_m) of polymer sample should be taken into account. The extraction efficiency of a polymer is enhanced above its T_g and is increased still further above the polymer T_m ⁴⁸. A temperature above the T_g results in enough molecular motion in the amorphous phase of the polymer so that the SF can diffuse into the region easily. However, a temperature higher than the T_m is not practical because once the crystalline phase of the phase melts, clogging the extraction system and possibly ruining the extraction vessel may easily happen. The LDPE sample used in this work has a T_g well below ambient temperature and its $T_m=106-115^\circ\text{C}$. Therefore, the highest temperature practical for this work was below 106°C .

As shown in **Figure 2.12**, the extraction efficiency at 450 atm increased considerably for Irganox 1076 and Irganox 1010 when the temperature was raised from

⁴⁷ Kurnik R.T., Holla S.J., Reid R.C. (1981) *J. Chem. Eng. Data* **26** 47

⁴⁸ McHugh M.A. Krukoni V.J. (1986) Supercritical Fluid Extraction: Principles and Practice, Butterworths: Boston

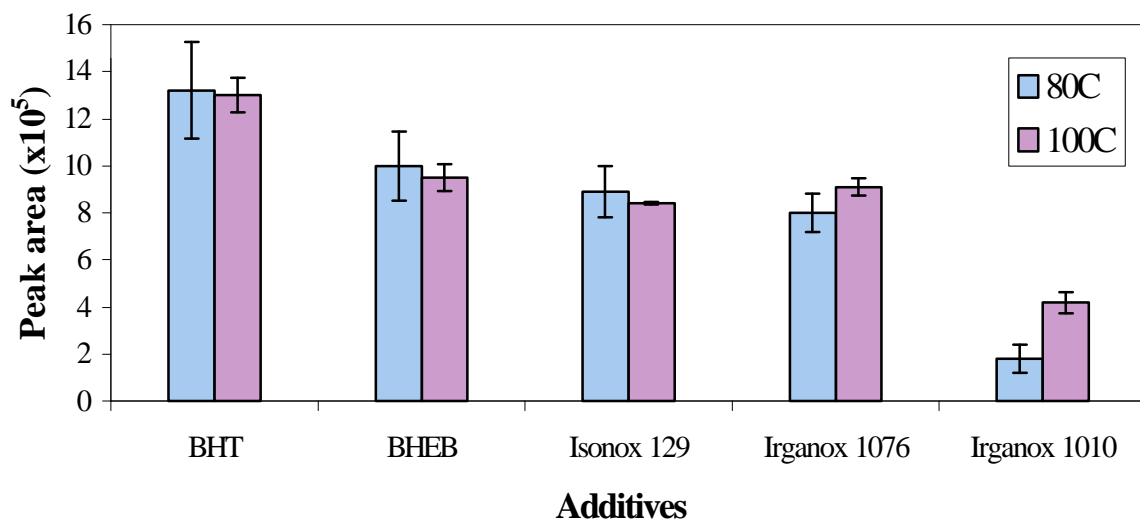


Figure 2.12. Effect of extraction temperature on extraction efficiency (Spiked sand).
 SFE conditions: 450 atm, 30 min, trapping at -40°C , flow rate: 0.65 mL/min.
 SFC/FID conditions: desorption at 180°C , pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C , DeltabondTM cyano, 100×1.0 mm, $5 \mu\text{m } d_p$, FID at 350°C .

80 to 100°C. Apparently, under these conditions, disrupting solute-solute interaction dominated the increase in the extraction of high molecular weight species in CO₂. Meanwhile, the extraction of BHT, BHEB, and Isonox 129 did not noticeably change with a temperature change, which suggested the extraction didn't vary very much with temperature at sufficiently high pressure due to their low molecular weight and small molecular size.

In the experiments described above, the pre-trap restrictor used was a piece of 25 cm × 25 µm fused silica capillary to afford a liquid fluid flow rate 0.65 mL/min. In order to investigate the effect of fluid flow rate on the extraction efficiency, the restrictor was replaced by a 50 µm fused silica capillary 25 cm long to obtain a higher fluid flow rate (i.e., 2.0 mL/min). A different extraction time was employed for the different fluid flow rates so that the amount of CO₂ used for each extraction was the same. The results are shown in **Figure 2.13**. Apparently, faster flow resulted in lower collection efficiency, because the extracted analytes easily vented out in the form of aerosol with the expanded CO₂ gas flow.

Experiments were also conducted to optimize the extraction time for the additive standards. For this purpose, 10 µL of the additive standard mixture was spiked onto the sand. The extraction was conducted at 450 atm and 100°C. The liquid CO₂ flow rate was 0.65 mL/min. The extracted components were collected at -40°C. The duration of each extraction was 10 min. As can be seen in **Figure 2.14**, the extraction of BHT and BHEB was completed in 10 min. No increase in the extraction yields of Isonox 129, Irganox 1076, and Irganox 1010 after a total 30 min extraction indicated that 30 min was sufficient time for the extraction of spiked sand.

In summary, lower trap temperature, higher extraction pressure, higher extraction temperature, and lower fluid flow rate with longer extraction time resulted in higher extraction efficiency. The optimum extraction conditions for spiked sand are reviewed below:

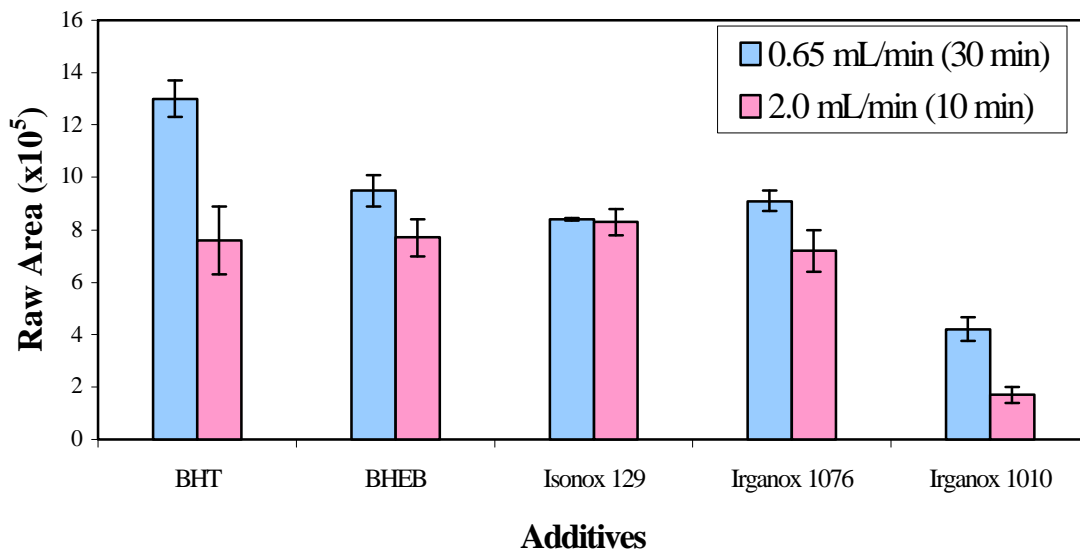


Figure 2.13. Effect of liquid flow rate on collection efficiency (Spiked sand).
 SFE conditions: 450 atm, 100°C, 30 min, trapping at -40°C.
 SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100 × 1.0 mm, 5 μm d_p, FID at 350°C.

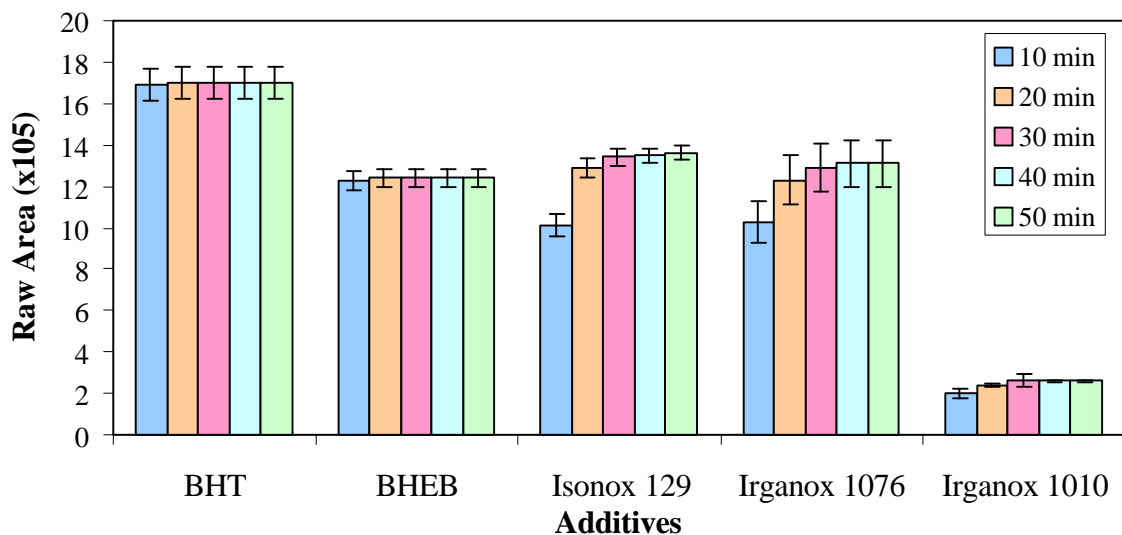


Figure 2.14. Effect of extraction time on extraction efficiency (Spiked sand).
 SFE conditions: 450 atm, 100°C, 10 min × 5, trapping at -40°C.
 SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100 × 1.0 mm, 5 μm d_p, FID at 350°C.

Pressure:	450 atm
Temperature:	100°C
Flow rate:	0.65 mL/min
Dynamic extraction time:	30 min
Trapping temperature:	-40°C

Creation of calibration curves for additive standards

Traditionally, there are several standardization techniques employed in the practice of chromatographic analyses, external standards, internal standards, and standard addition. Direct injection external calibration is sensitive to variations in the matrix, and therefore is unsuitable for many matrix systems due to the so-called matrix effect⁴⁹. Simply stated, the matrix effect is the manner in which the analytes interact with the sample matrix.⁵⁰ If the analytes or the matrix is changed, then the matrix effect also changes. Internal standards and standard addition can overcome the matrix effect, however, a homogeneous mixture of standard and sample is difficult to obtain if solid samples are analyzed⁴⁹. To overcome these problems, three point calibration curves of spiked sand with the additive standards were established in the range of 1-3 µg of each additive standard. Ten µL of the standard solution with various concentrations was applied via a microsyringe onto the sand in the extraction vessel. The solvent was evaporated at room temperature before the extraction vessel was pressurized. The extraction was conducted under the previously optimized conditions. Peak area counts versus the amount of the additive standards was plotted to provide a curve, the slope of which was an area response factor (area counts/µg additive) that could be compared directly to area counts observed in actual polymer samples.

Figure 2.15 shows the calibration curves of BHT, BHEB, Isonox 129, Irganox 1076, and Irganox 1010, respectively, as well as the correlation coefficients which were greater than 0.9. The numerical data are given in **Table 2.1**.

⁴⁹ Klaffenbach P., Bruse C., Coors C., Kronenfeld D., Schulz H. (1997) *LC/GC* **15**, 1052

⁵⁰ Markelov M., Guzowski J.P. Jr. (1993) *Anal. Chim. Acta* **276**, 235

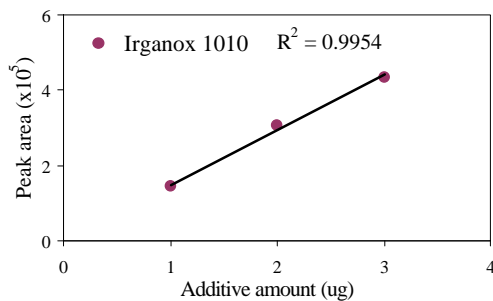
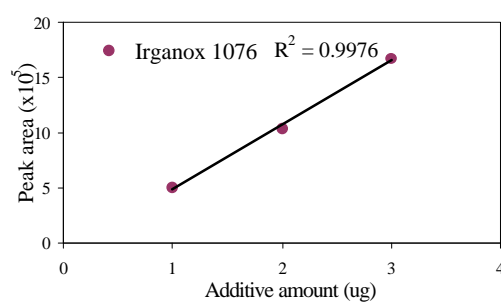
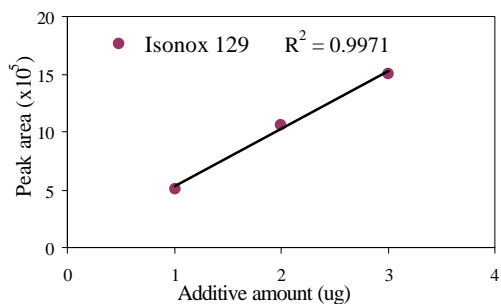
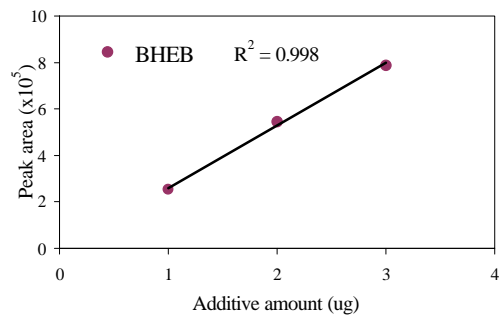
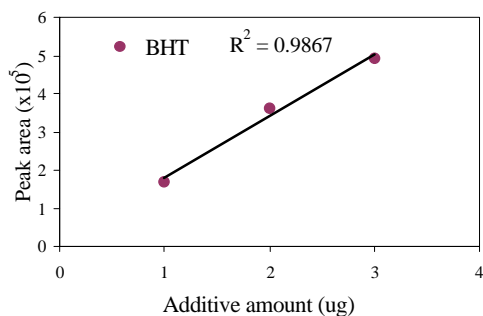


Figure 2.15. Calibration curves of additive standard from spiked sand.

SFE conditions: 450 atm, 100°C, 30 min, trapping at -40°C.

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100 \times 1.0 mm, 5 μm d_p , FID at 350°C.

Table 2.1. Peak area ($\times 10^5$) for calibration curves of spiked sand.

SFE conditions: 100% CO₂, 450atm, 100°C, 30 min, trapping at -40°C
SFC/FID conditions: desorption at 180°C, pressure programming:
100 atm for 3 min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at
100°C, Deltabond™ cyano 100 \times 1.0 mm i.d., 5 μ m d_p, FID at 350°C.

Additive amount	1 μ g	2 μ g	3 μ g
BHT	1.69 (4.5)	3.63 (5.8)	4.92 (11)
BHEB	2.53 (4.3)	5.42 (8.3)	7.89 (0.6)
Isonox 129	5.11 (14)	10.6 (2.9)	15.1 (4.8)
Irganox 1076	5.03 (11)	10.4 (5.3)	16.7 (1.8)
Irganox 1010	1.44 (3.6)	3.06 (1.7)	4.34 (3.1)

() indicates %RSD, n=3

Linearity of the calibration is very important. When the calibration is linear and passes through the origin, an area response factor can be calculated using a single point calibration.

Quantitation of additives from LDPE sample

The goal of this portion of the work was to quantitatively extract the additives from the LDPE sample under the previously optimized conditions for spiked sand. First, the extraction profile was obtained to determine the suitable extraction time for the polymer sample.

The LDPE sample provided by Quantum Corp.(Cincinnati, OH) was previously ground to 20 mesh, the large surface area is very important for transportation of the analytes to the bulk fluid⁵¹. Therefore the sample was extracted as it was received.

In order to make the extracted additive concentration fall into the linear range of the calibration curves obtained with spiked sand, approximately 2 mg of the LDPE sample was employed for each extraction. Another reason for the use of a small sample size was to avoid clogging of the small i.d. fused silica restrictor and overloading the column.

Figure 2.16 shows a representative on-line SFE/SFC chromatogram of the LDPE sample.

The extraction profile obtained for the additives from the LDPE sample using the optimized conditions is shown in **Figure 2.17**. It was found that the extraction of the relatively volatile and low molecular weight species such as BHEB was exhaustive in 15 min. This result suggested that the extraction of low molecular weight species was only solubility limited. However, for the extraction of the other three additives, Isonox 129, Irganox 1076, and Irganox 1010, which are nonvolatile and have higher molecular weights, the extraction profile was very typical of an extraction both solubility and diffusion limited. The initial extraction of the three additives occurred rapidly (in the first 15 min), which suggested that the extraction was dependent upon the solubility of the bulk

⁵¹ Bartle K.D., Clifford A.A., Hawthorne S.B., Langenfeld J.J., Miller D.I., Robinson R. (1990) *J. Supercritical Fluids* **27**, 143

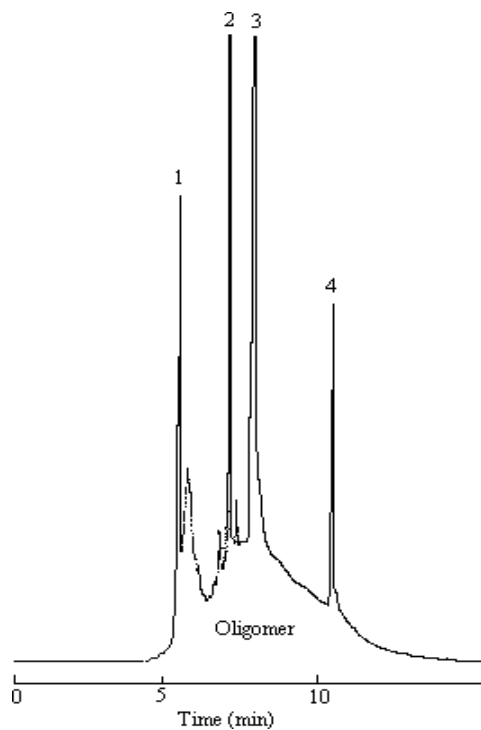
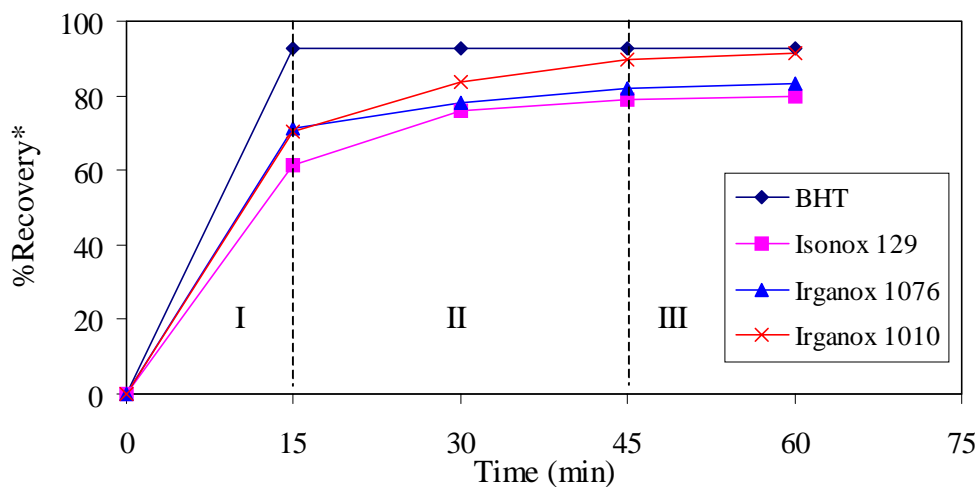


Figure 2.16. On-line SFE/SFC/FID chromatogram of LDPE sample (2.0 mg).

1. BHEB, 2. Isonox 129, 3. Irganox 1076, 4. Irganox 1010.

SFE conditions: 450 atm, 100°C, 15 min, trapping at -40°C.

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100×1.0 mm, 5 μm d_p, FID at 350°C.



* Mass extracted was determined via calibrations for spiked sand, %Recovery was calculated relative to vendor stated results.

Figure 2.17. Extraction profile of LDPE sample.

SFE conditions: 450 atm, 100°C, 15 min×4, trapping at -40°C.

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm for 3min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano, 100×1.0 mm, 5 μm d_p, FID at 350°C.

analytes in the SF (region I). Region II is an intermediate region where the extraction process was slower, which indicated that the extraction was enthalpically controlled (i.e., analyte-matrix interaction must be disrupted). A transition to diffusion limited kinetics also took place in this region. The extraction was much slower after 45 min (region III), which represented when the extraction process was truly diffusion-controlled¹. Therefore, for these three additives, 60 min was deemed a sufficient extraction time.

The percent recoveries under optimized SFE/SFC conditions of the additives from the LDPE sample are given in **Table 2.2**. The mass extracted was obtained from comparison with the calibration curves of spiked sand. The recoveries were calculated based on the original additive concentration provided by the manufacturer. Only BHEB, Isonox 129, Irganox 1076, and Irganox 1010 were quantified. BHT was not detected (ND), most likely due to the transformation of BHT radicals to dimer, which is not extractable¹⁸. **Figure 2.18** shows the transformation when dimerization of BHT occurs. Recoveries greater than 80% for each additive were achieved by the on-line SFE/SFC technique. However, the precision was quite low due to the very small sample size, approximately 2.0 mg. The inhomogeneous distribution of the additives in the polymer product could also result in low analysis precision.

Off-line SFE/HPLC

In the analysis using the on-line SFE/SFC system, a very small sample size was employed, which was believed to be the cause of the low precision. In this portion of the study, we tried to examine another technique, off-line SFE/HPLC, which can be used for large sample sizes.

A 500 mg sample of the LDPE, approximately 250 times that employed in the on-line SFE/SFC, was subjected to off-line SFE/HPLC. The extracts must be analyzed within 24 hours to avoid possible degradation of unstable species. **Figure 2.19** shows an off-line SFE/HPLC chromatogram of the extract of the LDPE sample. The additive percent

Table 2.2. Percent recovery of the additives from LDPE sample with on-line SFE/SFC.

SFE conditions: 100% CO₂, 450atm, 100°C, 60 min, trapping at -40°C,
sample size: 2 mg

SFC/FID conditions: desorption at 180°C, pressure programming: 100 atm
for 3 min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C,
Deltabond™ cyano 100×1.0 mm i.d., 5 μm d_p, FID at 350°C.

Mass extracted was determined via calibrations for spiked sand, %Recovery
was calculated relative to vendor stated results

	% Recovery (%RSD, n=3)
BHT	ND
BHEB	93(18)
Isonox 129	80(21)
Irganox 1076	83(18)
Irganox 1010	92(12)

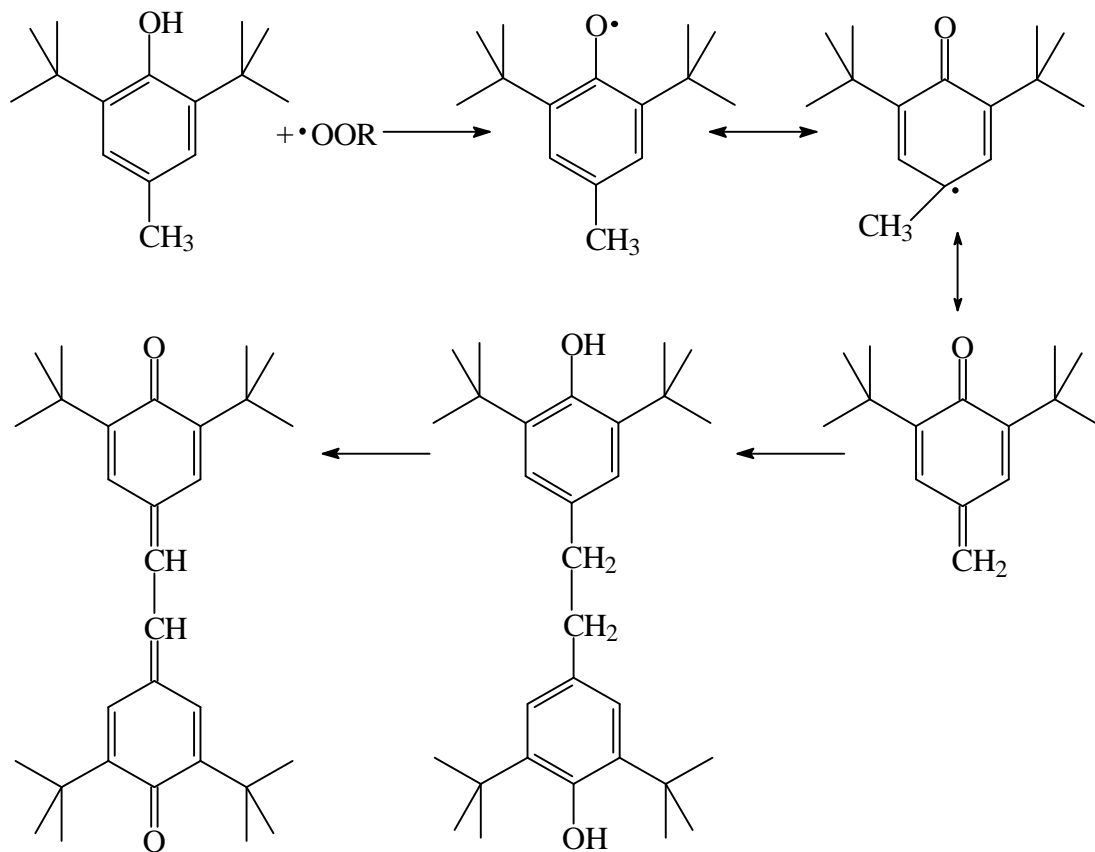


Figure 2.18. The transformation when dimerization of BHT occurs. (Taken from ref. 18)

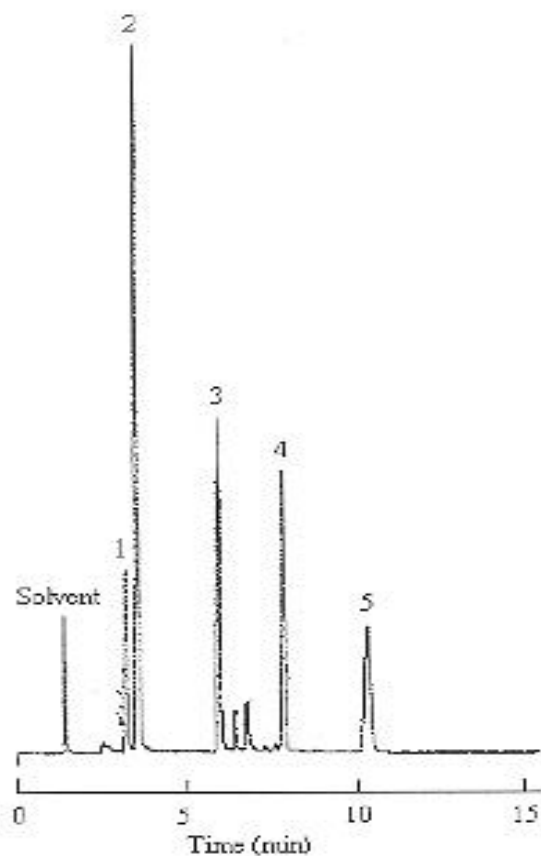


Figure 2.19. Off-line SFE/HPLC/UV chromatogram of LDPE sample (500mg).

1. BHT, 2. BHEB, 3. Isonox 129, 4. Irganox 1010, 5. Irganox 1076.

SFE conditions: 100% CO₂, 450 atm, 100°C, 30 min, ODS trapping at 0°C, flow rate: 1.5 mL/min, rinse with 5 mL ethylacetate/CH₃CN.

HPLC conditions: 150×3.9 mm, 5 μm d_p RP C₁₈, at 50°C, UV detector at 200 nm, mobile phase gradient from 75/25 (v/v) CH₃CN/H₂O to 100% CH₃CN in 5 min, hold 100% CH₃CN for 14 min, return to 75/25 (v/v) CH₃CN/H₂O at 19.01 min.

recoveries are given in **Table 2.3**, as well as those obtained from the on-line SFE/SFC. Lower recoveries were obtained with the off-line SFE/HPLC method because the coextracted oligomer could precipitate from the rinse solution and occlude a significant portion of the analytes. Heating the polymer extract solution would be helpful to dissolve the oligomer as well as the analytes. However, extreme care will be essential since some of the additives are thermally unstable. Higher recoveries were obtained with the on-line SFE/SFC method, most likely due to the fewer number of experimental steps and therefore the reduction in sample handling compared to the off-line SFE/HPLC. Also the use of the cryogenic collection trap eliminated the problem of the coextracted oligomer precipitating and occluding the analytes from the rinse solution. However, the presence of the coextracted oligomer from the SFE could render subsequent chromatographic integration deviation. This problem can be partially overcome by use chromatographic detectors having a specificity for the analyte of interest. In this case, a UV detection will be working. The recoveries from two techniques are comparable, except for Irganox 1076. However, the precision obtained from the off-line SFE/HPLC was much better compared to that from the on-line SFE/SFC. The different precisions obtained with the two techniques is believed to arise from the sample size difference, i.e., about 250 times amount of sample employed in off-line SFE/HPLC compared to the on-line SFE/SFC.

Off-line ESE/HPLC

Enhanced Solvent Extraction (ESE), also known as Accelerated Solvent Extraction (ASE™), is a new extraction method that significantly streamlines sample preparation. A commonly used solvent is pumped into the extraction cell containing the sample, which is then brought to an elevated temperature and pressure. Minutes later, the extract is transferred from the heated cell to a standard collection vial for clean-up or analysis. The entire extraction process is fully automated and performed in minutes for fast and easy extraction with low solvent consumption. ESE has been demonstrated to be equivalent to existing extraction methodologies such as Soxhlet and automated Soxhlet

Table 2.3. Percent recovery of the additives from LDPE sample.

On-line SFE/SFC: SFE conditions: 100% CO₂, 450atm, 100°C, 60 min, trapping at -40°C
SFC conditions: desorption at 180°C, pressure programming: 100 atm for 3 min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano 100×1.0 mm i.d., 5 μm d_p, Sample: 2.0 mg

Off-line SFE/HPLC: SFE conditions: 100% CO₂, 450 atm, 100°C, 30 min, ODS trapping at 0°C, rinse with 5 mL ethylacetate/CH₃CN at 25°C, sample: 500 mg; HPLC conditions: C₁₈ 150×3.9 mm, 5 μm d_p at 50°C, gradient from 75/25 (v/v) CH₃CN/H₂O to 100% CH₃CN in 5 min, hold 100% CH₃CN for 14 min, return to 75/25 (v/v) CH₃CN/H₂O at 19.01 min, flow at 1.5 mL/min, UV detector at 200 nm

	Off-line SFE/HPLC	On-line SFE/SFC
BHT	8(2)	ND
BHEB	104(8)	93(18)
Isonox 129	67(1)	80(21)
Irganox 1076	49(2)	83(18)
Irganox 1010	90(4)	92(12)

() indicates %RSD, n=3

for most RCRA (Resource Conservation and Recovery ACT) analytes from solid and semi-solid samples ⁵².

In our work, a 500 mg sample of LDPE was also subjected to the off-line ESE/HPLC process. **Table 2.4** and **Table 2.5** show the additive results in percent recovery and concentration (ppm), respectively. Comparable results were obtained with on-line SFE/SFC, off-line SFE/HPLC, and off-line ESE/HPLC, except for Irganox 1076. However, in ESE process, the tube easily clogged with the messy extracts. Therefore, clean-up of the system after the extraction was required.

⁵² Application Notes 324 (1996) Dionex Corp.

Table 2.4. Percent recovery of the additives from LDPE sample.

On-line SFE/SFC: SFE conditions: 100% CO₂, 450atm, 100°C, dynamic 30 min, trapping at -40°C SFC conditions: desorption at 180°C, pressure programming: 100 atm for 3 min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano 100×1.0 mm i.d., 5 μm d_p. Sample: 2.0 mg

Off-line SFE/HPLC: SFE conditions: 100% CO₂, 450 atm, 100°C, dynamic 30 min, ODS trapping at 0°C, rinse with 5 mL ethylacetate/CH₃CN at 25°C, sample: 500 mg; HPLC conditions: C₁₈ 150×3.9 mm, 5 μm d_p at 50°C, gradient from 75/25 (v/v) CH₃CN/H₂O to 100% CH₃CN in 5 min, hold 100% CH₃CN for 14 min, return to 75/25 (v/v) CH₃CN/H₂O at 19.01 min, flow at 1.5 mL/min, UV detector at 200 nm

Off-line ESE/HPLC: ESE conditions: 100% CO₂, 200 atm, 100°C, static 30 min, rinse with 10 mL ethylacetate/CH₃CN, flush with 10 mL CO₂, sample: 500mg; Same HPLC conditions as in off-line SFE/HPLC

	Off-line ESE/HPLC	Off-line SFE/HPLC	On-line SFE/SFC
BHT	8(5)	8(2)	ND
BHEB	104(10)	104(8)	93(18)
Isonox 129	68(2)	67(1)	80(21)
Irganox 1076	50(3)	49(2)	83(18)
Irganox 1010	94(16)	90(4)	92(12)

() indicates %RSD, n=3

Table 2.5. Concentration (ppm) of the additives from LDPE sample.

On-line SFE/SFC: SFE conditions: 100% CO₂, 450atm, 100°C, dynamic 60 min, trapping at -40°C SFC conditions: desorption at 180°C, pressure programming: 100 atm for 3 min, 100-330 atm in 7 min, 330-450 atm in 1.5 min, oven at 100°C, Deltabond™ cyano 100×1.0 mm i.d., 5 μm d_p. Sample: 2.5 mg

Off-line SFE/HPLC: SFE conditions: 100% CO₂, 450 atm, 100°C, dynamic 30 min, ODS trapping at 0°C, rinse with 5 mL ethylacetate/CH₃CN at 25°C, sample: 500 mg; HPLC conditions: C₁₈ 150×3.9 mm, 5 μm d_p at 50°C, gradient from 75/25 (v/v) CH₃CN/H₂O to 100% CH₃CN in 5 min, hold 100% CH₃CN for 14 min, return to 75/25 (v/v) CH₃CN/H₂O at 19.01 min, flow at 1.5 mL/min, UV detector at 200 nm

Off-line ESE/HPLC: ESE conditions: 100% CO₂, 200 atm, 100°C, static 30 min, rinse with 10 mL ethylacetate/CH₃CN, flush with 10 mL CO₂, sample: 500 mg; Same HPLC conditions as in off-line SFE/HPLC

	Manufacturer Data	On-line SFE/SFC	Off-line SFE/HPLC	Off-line ESE/HPLC
BHT	875	ND	67 ± 1	73 ± 4
BHEB	975	907 ± 163	1018 ± 81	1012 ± 101
Isonox 129	975	780 ± 164	650 ± 7	660 ± 13
Irganox 1076	1000	830 ± 149	488 ± 10	499 ± 15
Irganox 1010	975	897 ± 108	876 ± 35	913 ± 146

Conclusions

On-line SFE/SFC with cryogenic trapping was used to extract and separate five additives from a LDPE sample. The trap filled with glass wool was found to greatly improve collection efficiency. Spiked sand was employed to optimize the various parameters of the on-line SFE/SFC system. We found that lower trapping temperature (-40°C), higher extraction pressure (450 atm) and extraction temperature (100°C), lower fluid flow rate (0.65 mL/min) with longer extraction time (30 min) resulted in better extraction and collection efficiency. Higher desorption temperature (180°C) was essential to efficiently remove the extracted analytes from the trap to the separation column. The “Memory effect” of the on-line system and impurities in CO_2 tanks were studied. The use of the filter filled with activated carbon and adsorption alumina overcome the impurity problems. Suitable concentration is necessary to minimize the “memory effect”. Calibration curves of spiked sand of on-line SFE/SFC were obtained with good linearities for quantitation. Off-line SFE/HPLC and off-line ESE/HPLC were also performed to compare to the on-line SFE/SFC. The results obtained from on-line SFE/SFC were comparable to those from off-line SFE/HPLC and off-line ESE/HPLC, except for Irganox 1076. However, the precision obtained with on-line SFE/SFC was lower than that from off-line SFE/HPLC and off-line ESE/HPLC due to the small sample size employed in the on-line system. Using on-line SFE/SFC did minimize the sample handling and reduce the organic solvent used, i.e., no organic solvent was used in on-line SFE/SFC as compared to 35 mL of ethylacetate and CH_3CN used in off-line SFE/HPLC for each run. Despite the precisions lower than expected, on-line SFE/SFC method for quantitation of polymer additives are reliable and robust for application in routine analysis of quality control.

Vita

The author, Lucy Ying Zhou, was born on September 2, 1967 to Qianyuan and Qiuxia in Wuxi, China. She attended Jianxin high school of Qidong, China, graduating in 1984. She was awarded her Bachelor of Science degree in Chemistry from Fudan University of China in July of 1988. After graduation she worked in governmental and industrial departments in China. On March 31, 1991, she married Haiqing Yuan and on March 31, 1994 they had their first daughter, Amy Ruomei. She entered Virginia Tech in 1996 to pursue a Master of Science degree under the advisement of Dr. Larry Taylor.