Microwave-Assisted Extraction and Quantitative Analysis of High-Density Polyethylene Pellets Fortified with Flavors

W.M. Coleman, III and **L.A. Crumpler**
R.J. Reynolds Tobacco Company, P.O. Box 1487, Winston Salem, NC 27102-1487

Abstract

Microwave-assisted extraction (MAE) has been shown to be an easy, rapid, accurate, quantitative, and precise component of an overall method for the quantitative analysis of flavor components formulated into high-density polyethylene (HDPE) pellets. Under optimized extraction conditions, MAE can be perform extraction of flavors from pellets within ½ h with flavor recoveries ranging from approximately 90% to 100%. The variability in the data expressed as percent relative standard deviation from gas chromatographic–mass selective detector analysis of targeted flavor components is always less than 5%, indicating a precise method. In addition, the major components identified in the flavor formulation prior to formulation into the HDPE pellets are the major components detected in the extraction, indicating an accurate determination. Thus, MAE can be readily recommended as an essential component of a high-volume approach to the quantitative determination of flavors formulated into HDPE pellets.

Introduction

Microwave-assisted extraction (MAE) is a process of using microwave energy to heat solvents in contact with a sample in order to partition analytes from the sample matrix into the solvent. The ability to rapidly and reproducibly heat the sample solvent mixture is inherent to MAE and is the main advantage of this technique (1,2). In conventional heating, a period of time is needed to heat the vessel before the heat is transferred to the solution, however microwave energy heats the vessel more quickly. This keeps the temperature to a minimum and accelerates the speed of heating. Two types of microwave heating systems are commercially available for the analytical laboratory: an open- and closed-vessel system. By using closed vessels, the extraction can be performed at elevated temperatures and pressures, thereby accelerating the mass transfer of target compounds from the sample matrix to the extraction solution. A typical extraction procedure uses small volumes of solvents in the range of 10–30 mL and short extraction times of 15–30 min. These volumes and times are typically much smaller and shorter than those required by conventional extraction techniques. In most cases recoveries of the target analytes and reproducibility of results are improved compared with conventional techniques. Thus, MAE is an attractive alternative to conventional techniques and this is evident by the number of scientific papers published during the last few years (1).

Over the years, procedures based on microwave heating have replaced many of the conventional hot plate and other thermal-based techniques that have been used for decades in chemical laboratories. MAE applications have covered extractions of substances from biological materials and extend from analytical-scale to industrial-scale applications. The first application of MAE was performed in 1991 and dealt with the extraction of essential oils from plant products (3). Within this same time frame, research groups began to use MAE for the extraction of additives from polyolefins (4,5).

The principle of heating using microwave energy is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation (6). Ionic conduction is the electrophoretic migration of ions when an electromagnetic field is applied. The resistance of the solution of this flow of ions results in friction and thus heat. Dipole rotation means realignment of dipoles with the applied field. These two forced molecular movements result in heating. Polar molecules and ionic solutions, such as acetone and acids, absorb microwave energy strongly because they have a permanent dipole moment that is affected by the microwaves. However, nonpolar molecules such as hexane do not heat up when exposed to microwaves. Thus, in a number of instances, MAE is performed with solvent mixtures containing solvents with both high and low dipole moments, such as acetone and hexane. One of the most commonly used mixtures is hexane–acetone (1:1). Hexane does not heat in the microwave field, but, by mixing it with acetone,

* Author to whom correspondence should be addressed: email colemaw@rjrt.com.

heating takes place within a few seconds. This mixture of solvents has been determined to be very effective in removing additives from high-density polyethylene (HDPE) (2) . In an application note from CEM Corporation, an acetone–cyclohexane (70:30) solvent mixture was used very successfully in the quantitative recovery of additives to HDPE (6).

The current procedure employed for the analysis of the pellets for flavor content involves conventional extraction techniques requiring from 1 to 2 days for quantitative extraction, followed by analysis by gas chromatography (GC)–mass selective detection (MSD) (7) . In a similar fashion, flavors have traditionally been incorporated into "polymers" of selected types for the purpose of emulsion and encapsulation, and flavor constituents of natural products have been removed from the plant matrix by liquid extractions (8,9). Based on the presented information with that available in the literature, MAE appeared to be a viable alternative with which to substantially shorten the extraction time and hence make the extraction/sample preparation more compatible with high-volume, high-throughput analytical protocols. Thus, a series of experiments structured to evaluate the potential of MAE were performed and the results are presented herein.

Experimental

Standards/solvents

The four proprietary flavors were obtained from the Mane SA France, 620, Route de Grasse, 06620 Le Bar Sur Loup, and used as received. Target components of the flavors (ethyl-2-methylbutyrate, carvone, linalool and citral) were obtained from Aldrich Chemical Company (Milwaukee, WI) and used as received. Acetone and cyclohexane were obtained from Burdick and Jackson (Muskegon, MI). Acetone and cyclohexane are both volatile solvents, and exposure should be minimized by the use of the solvents in an approved fume hood.

Sample description/preparation/ extraction procedures

HDPE pellets containing preferred levels of selected flavors were produced and received from the R.J. Reynolds Tobacco Company, Research and Development Flavor Division (Winston Salem, NC) in sealed glass jars. The pellets were stored in sealed glass jars at –20°C and taken out only for a brief period of time in order to weigh known amounts for extraction. Four types of pellets were received, each containing a proprietary flavor labeled for identification purposes as flavors I, II, III, and IV.

Samples were prepared for extraction in the following manner: to a 100-mL glass microwave extraction vessel (Greenchem Type, CEM Corporation, Matthews, NC) containing a stirring bar was added 0.50 ± 0.01 g of pellets (~ 35 pellets) followed by 30 mL of a 70:30 (v/v) acetone–cyclohexane solvent. The vessel was then capped, sealed, and placed in a microwave-permeable holding container following the manufacturer's instructions. The container was then placed into the microwave oven (MarsX, CEM Corporation). Six individual samples of each pellet type were prepared at each set of extraction parameters. One of the containers was fitted with a special cap that allowed for the measurement of the extraction vessel temperature.

Following the completion of the extraction process and when the sample temperature was less than 40°C, the extraction containers were removed from the oven. The containers were vented by gently opening the sealed vessel to slowly release any pressure. Once at atmospheric pressure, the glass vessels were removed from the extraction holders and placed in a "test tube" rack. After swirling, approximately 4 mL of solution was taken from each tube via a disposable pipet. This solution was filtered through a Whatman (Clifton, NJ) 0.45-µm poly(tetrafluoroethylene) Autovial syringe filter into a 20-mL glass vial. From this glass vial was taken enough volume to fill a GC autosampler vial. The GC vial was capped and placed in a GC autosampler tray for analysis. Because of the volatility of the solvent solution, handling of extraction vessels was performed in an adequate fume hood.

The microwave operating conditions employed in determining the optimum extraction parameters for each flavor pellet were as follow: the instrument was a CEM MarsX; the instrument was operated in the extraction mode; the extraction vessel was 100-mL glass GreenChem (stirring at setting 3); the maximum power was 600 W; the percent power was 100%; the ramp to extraction temperature was 10 min; the extraction temperatures were 100°C, 115°C, and 130°C; and the extraction times were 10, 20, 30, and 45 min.

Standards preparation

Standards were prepared by dissolving known amounts of the neat flavors used to prepare the specific HDPE pellets in the

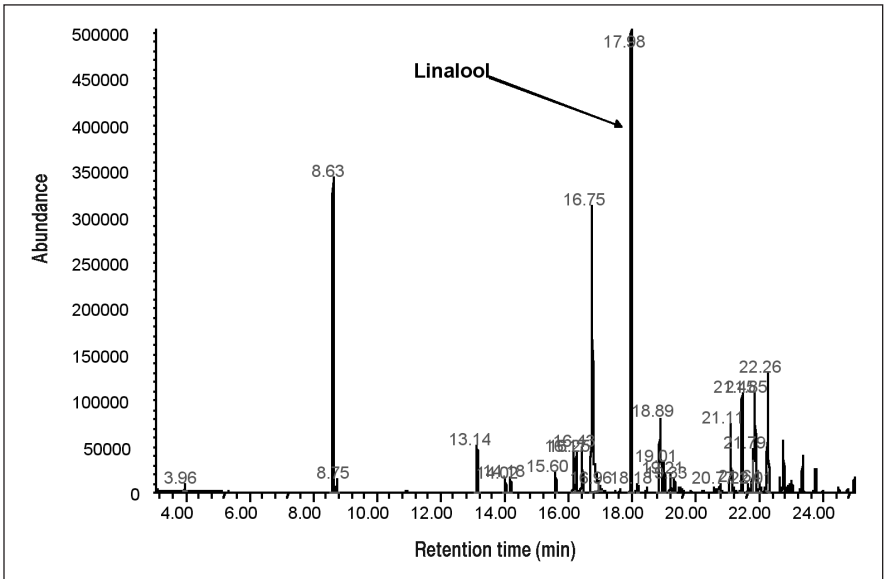

Figure 1. TIC of extract from Flavor Pellet I.

70:30 acetone–cyclohexane solvent. The desired concentration of each flavor solution was approximated through assuming 100% extraction efficiency from the MAE process. For example, the specification for the pellets with Flavor III called for 10% by weight flavor in the HDPE. Thus, a 0.5-g sample of these pellets would be expected to contain 0.05 g of Flavor III. After extraction, there should then be 0.05 g of Flavor III in 30 mL or 1666.67 µg/mL Flavor III in the extraction solvent. Thus, a standard solution of Flavor III of approximately 1600–1800 µg/mL was prepared. Standard solutions and neat flavors were stored at room temperature in sealed glass containers.

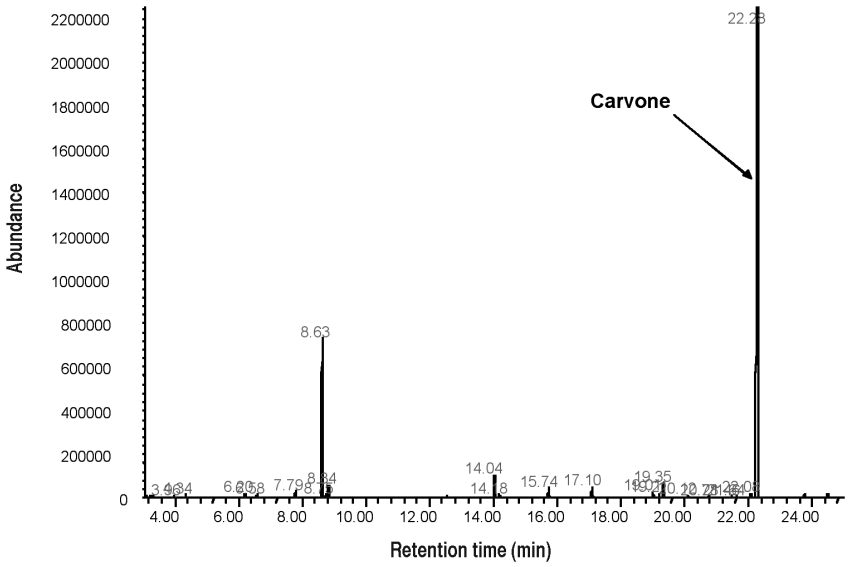

Figure 2. TIC of extract from Flavor Pellet II.

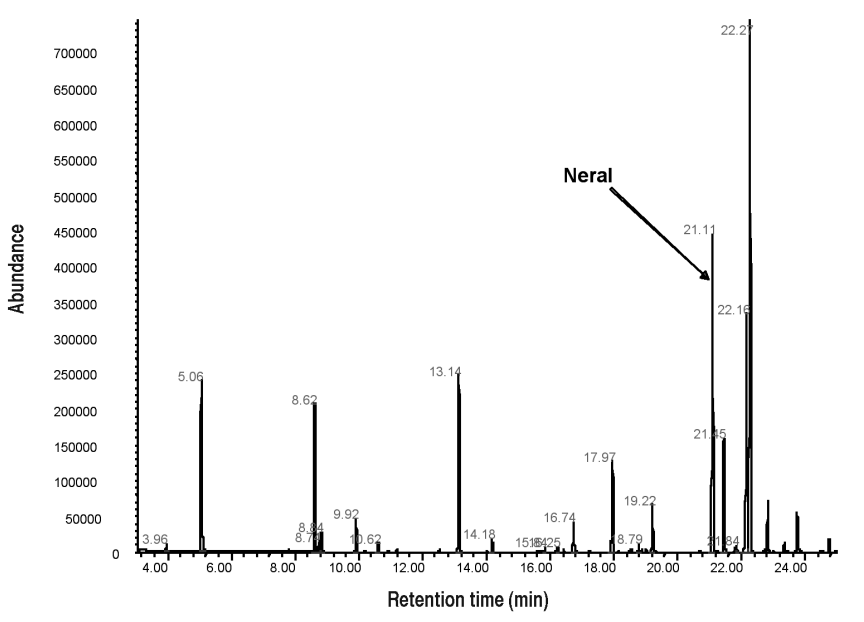

Figure 3. TIC of extract from Flavor Pellet III.

Table I. Target Compound and Calibration Data on Flavor Pellets

Flavor	Target analyte	Retention time (min)	Weight (%)	Calibration equation (R^2)
I	Linalool	17.98	15.82	$y = 83915x$, 0.989
II	Carvone	22.28	71.88	$y = 82303x$, 0.984
III	Neral	21.11	8.21	$y = 81008x$, 0.998
IV	Ethyl-2-methylbutyrate	5.11	100	$y = 62343x$, 0.987

Three of the flavors (I, II, and III) were found to be relatively complex mixtures via GC–MSD analysis (Figures 1–3). Rather than attempt to characterize the diverse number of analytes, one analyte was selected and targeted to represent the entire flavor. The selection of the analyte was made with two main criteria: (*i*) a relatively major component of the flavor, and (*ii*) the selected analyte represented the major sensory characteristic of the flavor. Thus, the target analytes for Flavor I, II, III, and IV were: linalool, carvone, neral, and ethyl-2-ethylbutyrate, respectively (Table I). Flavor IV was approximately 100% ethyl-2-methylbutyrate (Figure 4).

Because the target analytes represented only a portion of the flavor in three of the cases, the determination of the amount of target compound per unit mass of flavor was essential to the correct determination of recovery. To obtain these values, calibration curves of the targeted analytes were generated from prepared solutions of each target compound at known concentrations. Linear regression expressions with a zero intercept were calculated from the calibration curves. Based on the analyte response from the solution of the flavor, a concentration of the analyte in the flavor could be calculated, followed by a percentage analyte composition in the flavor (Table I). Therefore, a calculation of the expected amount/concentration of the analyte could be obtained assuming 100% extraction recovery.

GC–mass spectrometry analysis conditions

The analytical parameters were as follow: the system configuration was an Agilent 6890 GC equipped with a 5973 MSD and an autosampler; the column was a DB-WAXetr (30-m × 0.25-mm i.d., 0.25-µm film thickness) (J&W Scientific,

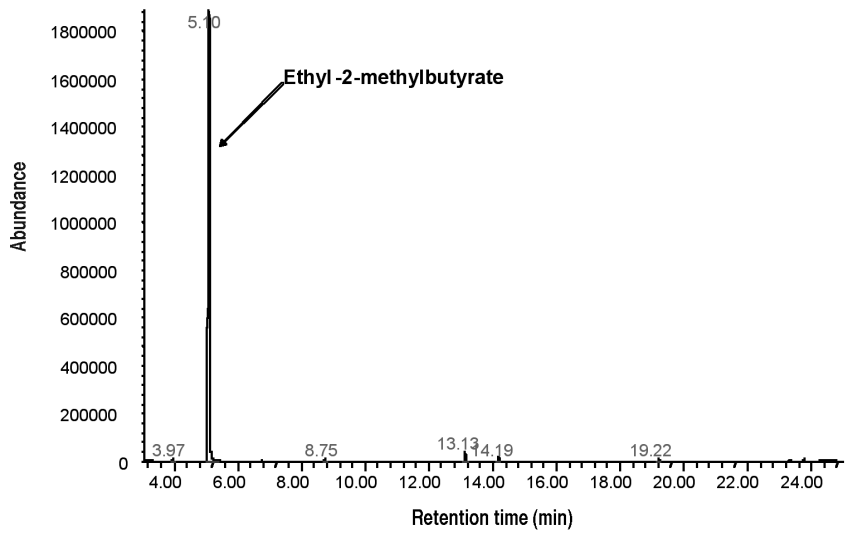

Figure 4. TIC of extract from Flavor Pellet IV.

Table II. Initial Extraction Conditions (X) Employed for Flavor Pellets

Temperature (°C)			
130	X		X
115		X	
100	X		X
Time (min)	10	20	30

Table III. Optimized Pellet Extraction Conditions for Target Analytes

Flavor pellet type	Extraction temperature (°C)	Extraction time (min)
I	115	20
II	100	30
III	115	20
IV	130	30

Table IV. Pellet Extraction Recoveries

Pellet type	Target analyte	Analyte TIC area (%RSD)	Amount Recovered (%)*
I	Linalool	2.02	105.3
II	Carvone	0.48	106.0
III	Neral	2.10	100.1
IV	Ethyl-2-methylbutyrate	1.02	89.5

* Expressed as a percentage of the measured amount of analyte extracted divided by the targeted load level.

Folsom, CA); the injection port temperature was 250°C, the injection was 1 µL, split 1/10; the inlet pressure was a constant flow at 1 mL/min; the column oven initial temperature was 45°C; the column oven initial time was 3 min and it was ramped 5°C/min to a final temperature of 155°C; the column oven final time was 0 min; the mass spectrometer (MS) transfer line temperature was 250°C; the MS quad temperature was 200°C; the MS source temperature was 250°C; the MS mass range was 33–300 *m/z*; the MS databases were NBS (November 1998, Wiley, New York, NY); and the MS configuration was selected ion monitoring at 70 eV.

The parameters were applied to the separation of each pellet type. The average of one injection of each sample from six individual extractions was used to calculate concentration and %RSD. Positive identifications of the analytes of interest in each flavor were made through a use of an internal retention time database as well as results from a Wiley Mass Spectral Library searches (Chemstation Software, Agilent Technologies, Wilmington, DE).

Results and Discussion

Optimization of extraction conditions

Based on the conditions appearing in the literature concerning the optimum extraction conditions for components in HDPE (6), a series of extraction experiments were conducted. The initial values for extraction time and temperature were varied slightly from the conditions presented in the literature (Table II). An "X" indicates that pellet extractions were performed at the set of conditions in an effort to obtain a maximum total ion current (TIC) response from the target analyte. Extensions in extraction time and temperature were employed should a local maximum not be obtained. Table III contains the optimum extraction conditions obtained for each pellet type.

General approach to sample analyte recovery calculations expressed as a percentage of the amount measured versus the amount loaded

Having established a set of extraction conditions for optimized extraction of the target analyte of each pellet type, calculations as to the % analyte recovery were made. The following is a step by step approach to the determination of the extraction recovery of the pellets with Flavor I, employing linalool as the target analyte. The approach can be applied to each pellet type with only a few modifications, *vide infra*.

From the calibration curve of average TIC area versus Concentration in µg/mL of linalool, an equation:

$$y = 883915x \qquad \text{Eq. 1}$$

with an R^2 of 0.989 is obtained from a linear fit. From a Flavor I solution, at 892 µg/mL in the extraction solvent, is obtained a linalool average TIC area of 11843936 with an RSD of 1.29%. Substitution of the value 11843936 for *y* in the calibration equation yields a concentration of linalool in the Flavor I of 141.14 µg/mL. Dividing 141.14 µg/mL by 892 µg/mL and multiplying by 100 yields a weight percent linalool in Flavor I of 15.82%. From 0.50 g of pellets containing Flavor I, at a 5 weight percent loading level in the HDPE, one would expect to have 0.025 g of Flavor I contained within the 0.5g of orange pellets. With 30 mL of solvent used and assuming a 100% recovery, then the concentration of Flavor I would be 0.025 g of Flavor I divided by 30 mL to yield a theoretical concentration of 833.33 µg/mL. With linalool at 15.82% of Flavor I, then one should have 833.33 µg/mL times 0.1582 or 131.83µg/mL of linalool, assuming 100% recovery. Injections of the six extracts of the pellets with Flavor I yielded an average linalool TIC area of 11648319 with a %RSD of 2.02%. Substituting 11648319 for *y* in the linalool calibration equation yields a linalool concentration of 138.81 µg/mL. Thus, dividing the obtained amount of linalool from the extraction (138.81 µg/mL) by the expected theoretical amount of linalool (131.83 µg/mL), yields a percent recovery of 105.3%.

Adjustments to general approach to sample analyte recovery calculations

For the pellets with Flavor IV, the target compound ethyl-2-methylbutyrate is essentially 100% of the Flavor IV, thus no percent composition calculation is required. For the pellets with Flavor III, no actual standard of neral is commercially available. Thus, citral is employed as a standard. Citral is a mixture of neral and geranial. Thus, one must first determine the amount of neral in citral. This is done by assuming that the TIC response per unit mass is identical for neral and geranial and that a percent contribution can be simply made by dividing the TIC area associated with neral by the sum of the TIC responses from neral and geranial. For the batch of citral provided in this case, the weight percent neral was 38.15%. Having the weight percent neral in citral now allows for the generation of a calibration curve for neral. The extraction efficiencies obtained under the optimized conditions listed in Table III for each pellet and using the flavor target analyte of interest are listed in Table IV.

Conclusion

Flavor components contained within HDPE pellets can be effectively extracted with MAE. The approach has been shown to be an easy, rapid, accurate, quantitative, and precise component of an overall method for the quantitative analysis of flavor components in the HDPE. More specifically, under optimized extraction conditions, MAE can perform quantitative extraction of selected flavor pellets within one-half hour with recoveries of approximately 90–100%. The variability in the data expressed as %RSD from GC–MSD analysis of targeted flavor components was always less than 5%, indicating a precise method. In addition, the major components identified in the flavor formulation prior to formulation into the HDPE pellets were the major components detected in the extraction, indicating an accurate determination. Thus, MAE is strongly recommended as an essential component of a high-volume approach to the quantitative determination of flavors formulated into HDPE pellets.

References

1. C.S Eskilsson and E. Bjorklund. Analytical scale microwave-assisted extraction. *J. Chromatogr. A* **902:** 227–50 (2000).
2. B. Marcato and M. Vianello. Microwave-assisted extraction by fast sample preparation for the systematic analysis of additives in polyolefins by high-performance liquid chromatography. *J. Chromatogr. A* **869:** 285–300 (2000).
3. J.R.J. Pare. Microwave-assisted natural products extraction. *U.S. Patent* 5002784, March 26, 1991.
4. R.C. Neilson. Extraction and quantification of polyolefin additives. *J. Liq. Chromatogr.* **14:** 503 (1991).
5. W. Freitag and O. John. Fast separation of stabilizers from polyolefins by microwave heating. *Angew. Makromol. Chem.* **175:** 181 (1990).
6. CEM Corporation. Microwave assisted solvent extraction, additives from high density polyethylene. CEM Application Note E012. CEM Corporation, Matthews, NC, 2003.
7. W.M. Coleman, III and L.A. Crumpler. R.J. Reynolds Tobacco Co., Winston Salem, NC. Unpublished room temperature solvent extraction data from authors' laboratory. 2003.
8. C.-T. Ho, C.-T. Tan, and C.-H. Tong. *Flavor Technology Physical Chemistry, Modification and Process.* ACS Symposium Series 610, American Chemical Society, Washington, D.C., 1995.
9. R. Teranishi, G.R. Takeoka, and M. Guntert. *Flavor Precursors Thermal and Enzymatic Conversions.* ACS Symposium Series 490, American Chemical Society, Washington, D.C., 1992.

Manuscript accepted February 20, 2004.