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Identification of a pharmaceutical packaging off-odor using solid phase microextraction gas chromatography/mass spectrometry

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Abstract

The use of a solid phase microextraction (SPME) sampling technique, in conjunction with gas chromatography/mass spectrometry (GC/MS) analysis, to identify an off-odor in a heat-stressed pharmaceutical packaging material is described. The ability of the commercially available polydimethylsiloxane (PDMS) coated microfiber to concentrate a trace volatile compound of interest enabled identification of the odor compound of interest. Despite being present at levels that defied detection using conventional headspace sampling techniques, ethyl-2-mercaptoacetate was determined to be the compound responsible for the offending odor. Formation of the thioester resulted from an unanticipated reaction (either esterification or transesterification) between a common residual solvent (ethanol), present in a commonly used pharmaceutical tablet dispersant, and low-level amounts of reactants or synthetic intermediates of an FDA-approved polyvinyl chloride (PVC)-resin thermal stabilizing agent.

Keywords: Solid phase microextraction; SPME; Gas chromatography; Mass spectrometry; GC/MS; Off-odor; Pharmaceutical packaging

1. Introduction

The occurrence of unexpected and undesirable odors emanating from packaging components has long been a matter of importance for manufacturers and processors of food [1–8] and beverages [9]. Most often, these odors originate from various additives in the polymeric packaging materials such as polyethylene (PE), polypropylene (PP) or polyvinyl chloride (PVC). These additives play a number of important roles, among them oxidative [10] and thermal stabilization [11] of the polymeric substrate. While these odors rarely, if ever, constitute cause for concern with respect to product safety, their impact on product desirability or acceptability by consumers can be significant. With the introduction of new packaging materials for use with pharmaceutical products,

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similar issues can arise. For the analytical chemist, charged with identifying or quantifying these odor compounds, the challenge is often daunting. The human olfactory sense can be exquisitely sensitive [12] and selective, in some instances surpassing the achievable limits of detection of modern analytical instrumentation. Compounding the difficulties encountered in such analyses are issues of representative sampling, sample handling or treatment, and choice of analytical methodology.

Classically, the investigation of an undesirable odor involves the analysis of a gaseous headspace using gas chromatography (GC). Headspace sampling [13,14] is carried out typically using a gastight syringe (direct injection), or by passing a volume of the headspace gas through some trapping medium (e.g. activated charcoal or some polymeric material such as Tenax™, with subsequent thermal desorption) [15,16]. These trapping techniques can significantly increase the concentration of the analyte(s) of interest. Detection can be accomplished either selectively or non-selectively in the GC analysis, using a variety of available detectors (e.g. flame ionization detection, thermal conductivity detection, electron capture detection, mass spectrometry (MS), etc.). Within the past few years, there have been several reports detailing the use of small, coated fibers [17–20] for selectively sampling and concentrating analytes of interest from both gaseous and liquid matrices. This technique of solid phase microextraction (SPME) has been used in conjunction with both GC and high-performance liquid chromatography (HPLC). Applications have included the analysis of a variety of pharmaceutical agents and their metabolites in biological fluids [21–27], environmentally important volatile organic compounds (VOC) in air and water [28–30], and the determination of volatile organic impurities (i.e. residual solvents) in pharmaceutical substances [31–33]. Recently, in our laboratories an objectionable odor was attributed to a commonly used packaging material for pharmaceutical tablet products (a PVC polymer-coated foil blister package). This report describes the use of SPME in conjunction with GC/MS analysis to identify the objectionable odor, which was detected after thermal stressing of this commonly available pharmaceutical tablet packaging material.

2. Experimental

The instrument used for GC/MS analyses consisted of a Hewlett Packard 5890 series II GC interfaced to a Hewlett Packard model 5972 mass selective detector (Hewlett Packard, Palo Alto, CA). The chromatographic capillary column used was a DB-5MS (J&W Scientific, Folsom, CA) having the following dimensions, 30×0.25 mm i.d.; 0.25-μm film thickness. The chromatographic elution was temperature programmed as follows: isothermal at 35°C for 5 min, then from 35 to 240°C at a rate of 10°C/min, then an isothermal hold at 240°C for 8.5 min. The column head pressure was set to 10 psi, and the carrier gas was helium. The injector and transfer line were maintained at 250 and 280°C, respectively. Mass spectra were acquired under electron ionization (EI) conditions in the m/z range of 35–500 at a rate of 1 scan per s.

A Supelco SPME fiber (sheathed in a stainless steel needle) was used for sampling. The stationary phase had a film thickness of 100 μm and was composed of polydimethylsiloxane (PDMS). The steel needle containing the PDMS fiber was inserted through the septum of the sample vial in order to sample the headspace for 30 min. After exposure, the PDMS fiber was retracted into the steel needle and removed from the sample vial. The steel needle was then pushed through the septum of the GC injection port and the PDMS fiber was extended, exposing it for 2 min to the 250°C temperature of the GC injection port.

Ethyl-2-mercaptoacetate [623-51-8] was obtained commercially (Lancaster, Windham, NH) and used without further purification. The tablet dispersant sodium carboxymethyl starch (Explotab®, [9063-38-1]) was obtained from Penwest Pharmaceuticals (Patterson, NY).

The packaging material used in this study was cold form blister material (OPA/foil/PVC with PVC as product contact) obtained from Algroup Wheaton (Pharma Center Shelbyville, KY). The foil pieces measured approximately 72 in.^{2} in area.

They were placed in pre-cleaned 40-ml glass vials (Qorpak Corporation, Bridgeville, PA), sealed with teflon-faced silicon septa containing plastic screw caps (also from Qorpak Corporation) and stored for 2 weeks at 60°C. The reaction time and temperature (in excess of ICH conditions) were chosen to maximize production of the compound of interest and thus facilitate the qualitative identification effort. Containers were cooled and the headspace sampled using the microfiber as described above.

2.1. Results and discussion

Registration of pharmaceutical products for commercial sale requires extensive testing to ensure both the safety and efficacy of the finished product. Implicit in the determination of product safety is the physical and chemical stability of the total drug formulation and its package (i.e. stability and compatibility of drug substance with excipients, as well as compatibility with packaging components). Together, these factors impact expiry dating (approved shelf life) for the marketed product.

During the course of compatibility testing (under thermal stress conditions) an off-odor was detected when tablets were removed from their package (cold-form foil blisters constructed of aluminum foil and PVC, with push through lidding) for assay. Packaged tablets (active and placebo) had been stressed at 25°C/60% relative humidity (RH), 40°C/75% RH, and 50°C/ambient conditions. All of the samples exhibited a distinct and recognizable organosulfur (e.g. thiol, sulfide, etc.) odor. Samples stored for 1, 3 and 9 months all exhibited the odor upon perforation of the blister lidding. Based on the different stability storage conditions and time intervals, a correlation was established between odor intensity and time/temperature. Humidity did not appear to be a factor. Because the presence of the odor could have a potential impact on product shelf life, an investigation into its cause was initiated.

The various tablet formulation and packaging components were prepared and stored at elevated temperature to ascertain which components (either individually or in combination) were responsible for the source. This experimental matrix ruled out the tablet active ingredient and many of the excipients, since they generated no odor when stored in the absence of packaging materials. However, the combination of one particular tablet excipient (the tablet dispersant Explotab®) and the PVC-coated foil did exhibit the undesirable odor upon removal from the oven. The distinct odor (organosulfur in nature) was indistinguishable from that detected in the original stability samples. In order to identify the offending compound, GC/MS was selected as the analytical method of choice because of its excellent sensitivity and qualitative identification capabilities.

Initially, the typical approach of direct headspace sampling was attempted using a standard gas-tight syringe. Because the levels of analyte were expected to be very low, a 1-ml volume of headspace was injected into the GC. The results of that analysis (Fig. 1) failed to produce any recognizable response for the expected sulfur-containing compound (i.e. no indication of either the presence or identity of the offending odor).

In order to increase the amount of volatile compound(s) of potential interest being introduced into the GC, the experiment was repeated using the solid-phase micro extraction fiber. This recently available device has shown promise in similar applications. The headspace of the glass vessel containing the pieces of packaging material foil and the excipient was again sampled following the procedures described previously. Desorption of the fiber in the injection port of the GC, followed by temperature programming yielded the total ion chromatogram shown in Fig. 2 (Upper panel). Fig. 3 (Upper panel) shows an expansion of the 6–7.8 min region of Fig. 2. The difference in overall appearance of the two GC traces (compare Figs. 1 and 3) is quite striking. Desorption of the microfiber produced a chromatogram exhibiting numerous responses. Judging from the change in signal intensity (increased by more than two orders of magnitude), it was clear that the microfiber effected a considerable concentration of the volatile headspace compounds in the sampling vessel. While this increase in the absolute amount of analytes being introduced into the GC was advantageous for low-level detection, the interfer-

ing compounds being emitted from the stopper under the thermal stress conditions largely obscured the odor compound(s) of interest. These interferences were present despite attempts to minimize their appearance by selecting stoppers of high thermal stability and of compositions expected to minimize the number and amount of volatile compounds. It had been anticipated that the microfiber would add some degree of selectivity to the sampling process, but this hope was not realized in this instance. Fig. 2 (Lower panel) shows the total ion chromatogram generated from the headspace of the sampling vessel after heating without foil material/excipient contents (blank).

While the lack of selectivity in the sampling process was somewhat disappointing, the use of MS as the detection scheme for the analysis enabled the analyte of interest to be detected quite readily. The use of GC/MS was considered originally so that the offending odor compound(s) could readily be identified once separated by the GC. The nature of the odor component(s) of interest (i.e. distinctly recognizable as organosulfur compounds) in combination with the unique MS fragmentation behavior of such compounds under electron ionization conditions, made target compound detection possible. Thus, while the total ion chromatogram indicated a significant number of compounds present in the headspace of the thermally stressed foil/excipient combination (Fig. 2, Upper panel), selected ion traces (mass chromatograms of m/z 33, 47 or 61) for signals unique to sulfur-containing molecules generated a single chromatographic response (Lower panel of Fig. 3 shows the m/z 47 mass chromatogram). The full EI mass spectrum obtained for the chromatographic peak (suspected odor compound) at approximately 7.1 min is shown in Fig. 4.

Under EI conditions, most organic molecules undergo extensive fragmentation, generating ions

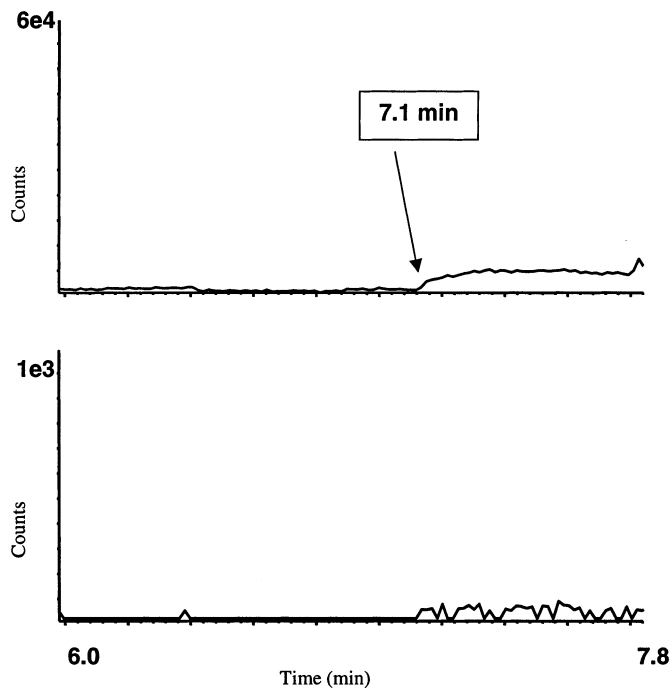

Fig. 1. (Upper panel) Expanded region of EI total ion chromatogram (TIC) obtained for the headspace from a sampling vessel using conventional gas-tight syringe sampling technique. The sample vessel contained the PVC-coated foil cuttings and the Explotab® tablet excipient (compare with Upper panel of Fig. 3). Expected elution time of the odor compound is noted. (Lower panel) Mass chromatogram (m/z 47) displayed over the expanded elution region of the analysis performed using the conventional sampling techniques (compare with Lower panel of Fig. 3).

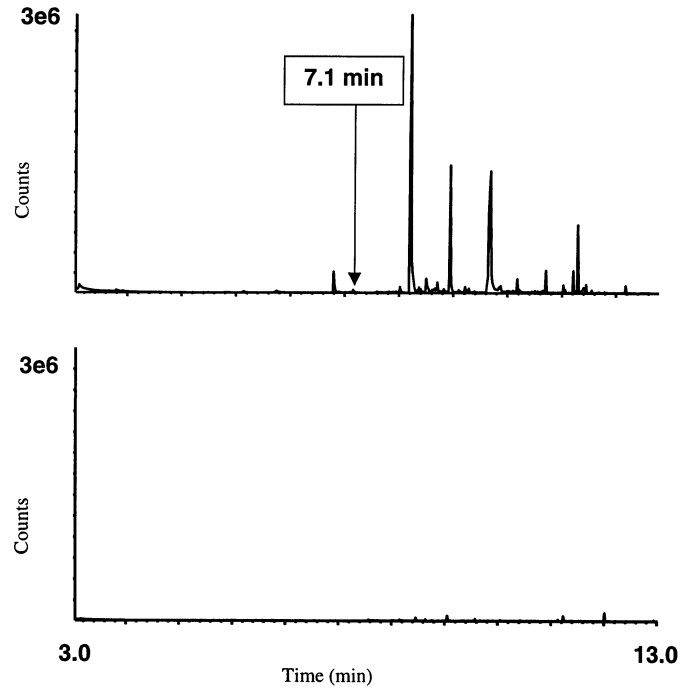

Fig. 2. (Upper panel) Electron ionization (EI) TIC of the headspace from the sampling vessel using SPME sampling technique. The sample vessel contained the PVC-coated foil cuttings and the Explotab® tablet excipient. Elution time of the odor compound is noted. (Lower panel) Total ion chromatogram of the headspace from an empty sample vessel using the SPME fiber sampling technique (blank).

representative of various functional groups and other structural elements. For organosulfur compounds (mercaptans, sulfides, etc.), depending on the size of the molecule and the respective substituents, ions with m/z 33 ($[HS]^+$), m/z 47 ($[HS{=}CH_2]^{+\cdot}$) and m/z 61 ($[HS{-}CH{-}CH_3]^+$) are commonly observed in their EI mass spectra. Since these ions are unique to this compound class, selected ion traces for these masses will be highly specific. Thus, the chromatographic response observed in the selected ion traces (mass chromatograms) of these unique ions, in combination with the characteristic nature of the odor being detected by the human olfactory sense, gave a good indication that the analyte of interest had been identified. Once distinguished from the chemical background of the headspace, the EI mass spectrum (background subtracted, Fig. 4) for this compound was obtained easily and matched with a reference mass spectrum from the searchable spectral library. The library match indicated that the odor compound of interest was ethyl-2-mercaptoacetate. To confirm this tentative identification, authentic compound was obtained from a commercial source, and analyzed. The GC retention time, the EI mass spectrum, and the distinct odor of the authentic material were all identical to that of the analyte detected originally in the tablet packaging material.

Because the synergy between the tablet dispersant (Explotab®) and the PVC-coated foil was essential to the production of the odor compound (neither material produced the odor when thermally stressed individually), we examined that relationship in order to understand fully the genesis of the ethyl-2-mercaptoacetate. A survey of the literature revealed that PVC polymer resins generally contain various additives designed to enhance pliability, as well as improving oxidative and thermal stability [34]. One of these common heat stabilizing additives is an organotin compound, di-n-octyltin-bis(i-octylthioglycolate). Among the

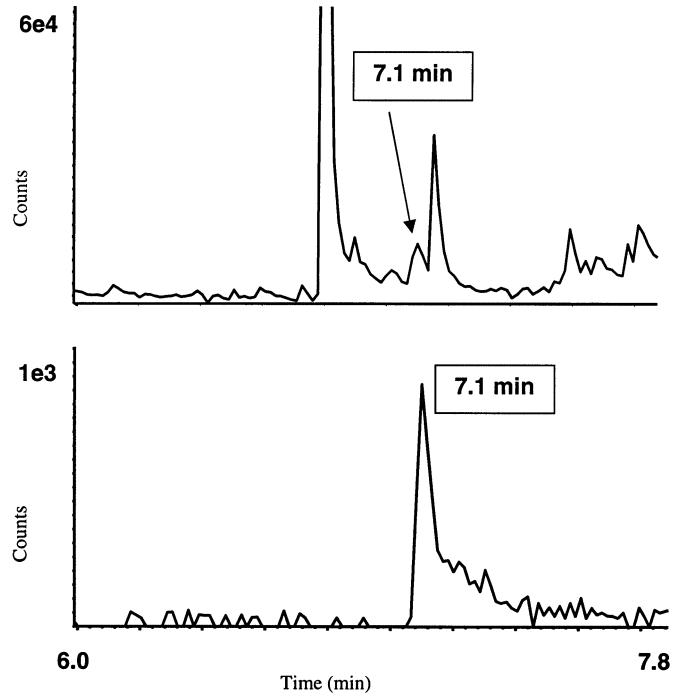

Fig. 3. (Upper panel) Expanded region of the TIC from Fig. 2 showing the elution region for the odor compound (ethyl-2-mercaptoacetate) sampled using the microfiber. (Lower panel) Mass chromatogram (m/z 47) displayed over the expanded elution region of interest. The specificity of the m/z 47 ion for the compound of interest is demonstrated by the absence of the other responses observed in the TIC.

reactants and synthetic intermediates of this FDA-approved packaging additive are thioglycolic acid and i-octylthioglycolate. Discussions with the packaging manufacturer confirmed the material composition described in the literature. Given the potential existence of one or both of these compounds in the PVC resin of the cold-form foil blister packaging components (due either to incomplete reaction or possible degradation), it then requires only the presence of ethanol (in this specific case) or some other low-molecular weight alcohol to produce a volatile thioester (either through esterification or transesterification), and hence an objectionable odor. Residual solvent analysis (using either headspace GC/FID or GC/MS) of the Explotab® had revealed the presence of ethanol. Thus, all the necessary conditions for production of the ethyl-2-mercaptoacetate existed in the tablet and placebo blister packages.

Confirmation of the formation mechanism was carried out by heating samples of the suspect lot of PVC-coated foil (used in the fabrication of the blister packages that produced the odor) with a

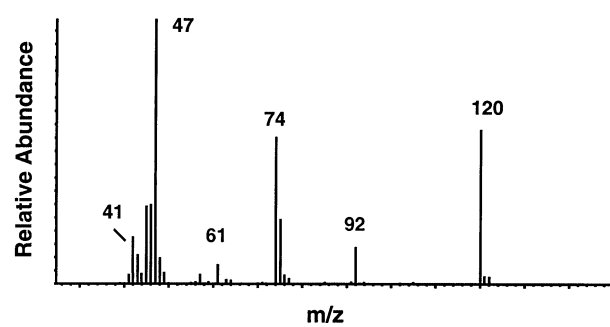

Fig. 4. Electron ionization (EI) mass spectrum of the 7.1 min peak in the chromatogram shown in Figs. 2 and 3. This spectrum is identical to that obtained for authentic ethyl-2-mercaptoacetate (not shown). Chromatographic retention times for the peak obtained from the sample and that for the authentic standard were identical.

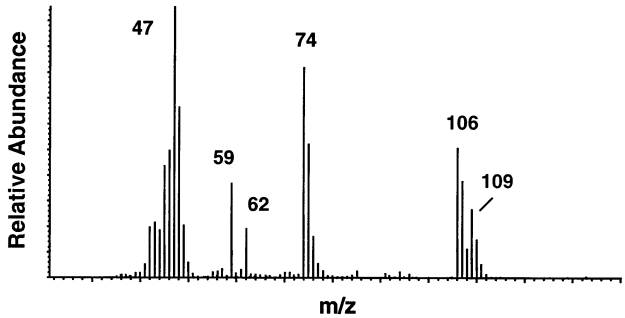

Fig. 5. Electron ionization (EI) mass spectrum of the methyl ester analog produced upon heating of the suspect packaging materials with a 50:50 mixture of methanol/methanol-d_3. The presence of isotopomers is clearly indicated by the distribution of signals (multiplicity) in the mass spectrum.

50:50 mixture of methanol/methanol-d_3. Methanol was selected because it would produce an analogous thioester (methyl-2-mercaptoacetate) and plays no part in the manufacturing process of the Explotab® (ensuring no ambiguity in the source of the reacting alcohol). The dueterated analog, methanol-d_3, was added to make detection by MS unequivocal (the isotopic pattern of the 50:50 mixture of the d_0:d_3 esters would be unmistakable). As expected, the reaction vessel containing the foil and methanol developed the same unmistakable odor upon heating, and GC/MS analysis verified the presence of methyl-2-mercaptoacetate/methyl-d_3-2-mercaptoacetate (Fig. 5). While neither thioglycolic acid nor i-octylthioglycolate were detected by GC/MS, their relatively low volatility (with respect to the low-molecular weight ethyl ester) and exceptionally low concentration could easily account for this fact.

3. Conclusions

Solid phase microextraction was used in conjunction with GC/MS analysis to identify an off-odor emanating from a commonly available pharmaceutical tablet packaging material (heat stressed). The distinct nature of the odor compound made identification important so that the information could be provided to the packaging manufacturer. The ability of the SPME fiber to adsorb and concentrate the analyte of interest enabled the analysis of a compound present at low levels. The limitations of the technique revolve around matching fiber coating composition with analyte compound class. For samples that contain a variety of analytes having disparate chemical compositions, a single fiber coating composition will discriminate against some of the compounds. Similarly, fiber performance is optimal when analyte volatility is matched with optimal fiber coating thickness. Again, if a complex sample contains a variety of components that possess a range of volatilities, choosing single fiber coating thickness will inevitably represent a compromise situation in terms of optimum performance. However, the technique has demonstrated considerable versatility in analyzing numerous classes of analytes in a variety of matrices and has in many ways revolutionized sample preparation and introduction techniques.

While rigorous quantification was not attempted in this investigation, the selectivity and sensitivity of the human olfactory sense for this and other organosulfur compounds (in conjunction with serial dilutions used for the preparation of qualitative standard solutions and associated rudimentary calculations) leads us to believe that the analyte of interest was present at 1 ppm or less in the headspace of the sampling vial. The source of the odor was traced to the unanticipated reaction of a common residual solvent in a widely used pharmaceutical tablet excipient with low-level residual amounts of reactants or synthetic intermediates of an FDA-approved PVC-resin thermal stabilizing agent.

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