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Investigation of mass-balance issue in e-beam sterilized paclitaxel eluting coronary stents by SPME/GC–MS

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ABSTRACT

Paclitaxel eluting coronary stents were sterilized by e-beam in a closed system, to investigate sterilization related mass-balance issues and evaluate potential volatile paclitaxel degradation products. A solid-phase microextraction (SPME) method utilizing a polydimethyl-siloxane/divinyl-benzene (PDMS/DVB) fiber was optimized for extracting the volatiles from the head-space of the sterilized stents. GC–MS was used for separation, identification, and quantitation of the components. Benzaldehyde and benzoic acid were identified as paclitaxel related volatile degradation products. Three groups of stents were included in the study, a control group (not exposed to e-beam), a group sterilized at 25 kGy, and a final group sterilized at 75 kGy. The stents sterilized by e-beam at 75 kGy contained significantly higher levels of benzoic acid relative to the controls and the stents at 25 kGy contained intermediate levels of benzoic acid. The benzaldehyde levels increased in the 25 kGy e-beam sterilized stents relative to the control but remained fairly constant in the 75 kGy e-beam sterilized stents relative to the 25 kGy e-beam results. Mechanism for the formation of benzoic acid and benzaldehyde from paclitaxel was proposed. The levels of benzoic acid and benzaldehyde observed on the stents did not resolve the original mass-balance issue, but most likely contribute to the lack of mass balance observed for paclitaxel.

1. Introduction

Paclitaxel (Taxol® is a registered brand name of Bristol-Myers Squibb) is a highly functionalized diterpene member of the taxane class of compounds. Based on the mode of action of paclitaxel it has been used in drug-eluting coronary stents to prevent cardiovascular restenosis [1]. Drug-eluting coronary stents are coated or filled with different drug/polymer mixtures and the polymer typically controls the drug release after implantation. Paclitaxel stability and degradation under various stress conditions have been studied and documented in the literature [2,3]. Paclitaxel degradation occurs primarily when exposed to basic conditions, hydrogen peroxide (oxidation) and high intensity light [2]. Chemical degradation under basic conditions involves hydrolysis of the ester linkages and formation of 10-deacetyl paclitaxel, 7-epipaclitaxel, and baccatin III as major degradation products. Paclitaxel can form numerous light induced degradants due to the formation of free radicals, which are stabilized by skeletal rearrangements [2]. Paclitaxel shows minimal degradation under acidic and oxidative conditions with the major degradants being 10-deacetyl paclitaxel and the water adduct of paclitaxel. No studies were found in the literature that reported the formation of volatile degradants of paclitaxel.

The Conor Medsystem CoStar® drug-eluting stent combined paclitaxel in a matrix of poly(lactic-*co*-glycolic acid (PLGA)). It is well known that polymers can be chemically altered and their properties impacted by exposure to ionizing radiation [4–7]. The effect of ionizing radiation on the stability and performance of the Taxus Express paclitaxel eluting stent was studied and reported [8]. The effect of ionizing radiation on polymers is generally divided into main-chain scission (degradation) and cross-linking (polymerization). Polymer degradation, where chain scission is dominant, is associated with a molecular weight (Mw) reduction and decreasing of polydispersity index.

In order to extract and analyze volatile components from the paclitaxel eluting stent, solid-phase microextraction (SPME) followed by analysis by gas chromatography–mass spectrometry (GC–MS) was used. SPME is routinely used in combination with gas chromatography (GC) and GC–MS and has been successfully used for the analysis of a wide variety of compounds [9]. More specifically, the use of SPME as a flexible and highly effective extraction method was successfully implemented in the pharmaceutical industry as described in recent review articles [10,11]. Since SPME as an extraction and sample concentration technique is readily adapted to analysis by GC–MS instrumentation SPME/GC–MS methodology has been intensively developed to extract and identify

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unknown volatile residual solvents in different pharmaceutical matrices [10,12]. More recent articles describe SPME extraction methods for active pharmaceutical ingredient related compounds [11,13].

Prior HPLC and LC–MS analysis of Conor Medsystem CoStar® paclitaxel eluting coronary stents analyzed post-e-beam sterilization showed similar degradation for paclitaxel as was previously reported in the literature [2]. However mass balance for paclitaxel was not obtained in the analysis [14]. Analysis of the paclitaxel loss observed post-e-beam sterilization of the stents was not explained by HPLC or LC–MS data. One of the possible reasons for the paclitaxel loss was a high degree of degradation and formation of volatile compounds that were lost during sterilization, since sterilization occurred in an open environment. Additionally, localized temperatures can be elevated significantly above ambient temperatures during sterilization increasing the potential for volatile loss. This study investigates the hypothesis that the lack of paclitaxel mass balance was caused by loss of volatile degradants during e-beam sterilization.

2. Experimental

2.1. Materials

CoStar® 2.5 mm × 22 mm paclitaxel eluting coronary stents were received from Conor Medsystem. The CoStar® coronary stents are designed with nanoliter size holes in the bare metal stent. During the manufacturing process those nanoholes are filled with a filling solution of paclitaxel and poly(lactic-*co*-glycolic acid) in DMSO. Once the holes are filled, the stents are dried, packaged and sterilized by e-beam at 25 kGy. For this study, single stents were placed into a Teflon™-valve (Supelco, Bellefonte, PA, USA) sealed 4-ml borosilicate glass vial (Supelco, Bellefonte, PA, USA) directly after the manufacturing drying procedure, prior to sterilization. (The borosilicate glass vial is designed to perform SPME extraction post-sterilization without opening the vials. This system was used in order to trap potential volatile compounds formed during sterilization.) The stents were grouped into three different groups. One group was unsterilized and used as the control sample. The next group of stents was sterilized with 25 kGy e-beam and the third group were sterilized with 75 kGy e-beam. Samples were stored below 0 °C post-sterilization prior to analysis. Benzaldehyde [CAS: 100-52-7], dimethyl-sulfone [CAS: 67-71-0], benzoic acid [CAS: 65-85-0], and dichloro-methane [CAS:75-09-2] were purchased from Sigma–Aldrich (St. Louis, MO, USA). 75/25 poly(lactic-*co*-glycolic acid) (Mw = 42 kDa) polymer was purchased from Durect Corporation (Birmingham Division, Pelham, AL, USA) for preparation of the polymer matrix used to prepare calibration curves.

2.2. Apparatus

The SPME fiber optimization experiments were performed with a manual fiber holder and fibers obtained from Supelco (Bellefonte, PA, USA). Three different fibers were selected for the optimization process; 100 μm non-chemically bonded polydimethyl-siloxane, for extracting low molecular weight most apolar volatiles (Supelco Bellefonte, PA, USA), 65 μm chemically bonded polydimethyl-siloxane/divinyl-benzene composite polymer coating for extracting low Mw, low and medium polarity components (Supelco Bellefonte, PA, USA) and 75 μm non-chemically bonded Carboxene/PDMS (activated charcoal/ polydimethyl-siloxane), for extracting the most volatile components (Supelco Bellefonte, PA, USA). For quantitation and for performing the calibration curves the PDMS/DVB extraction fiber [15,16] was selected. The samples were extracted using a MPS-2 automated SPME sampler (Gerstel, Wilmington, DE, USA).

An Agilent 6890/5975 inert GC–MS system (Wilmington, DE, USA) with parallel flame ionization (FID) detection was used for the analysis. Data acquisition was performed with Agilent ChemStation version D.02.00.275 (Wilmington, DE, USA).

2.3. GC–MS conditions

A J&W DB-5 ms fused silica capillary column (40 m × 0.18 mm with 0.18 μm 5% phenyl–95% dimethyl-silicone chemically bonded film) was used to separate the compounds. A split/splitless injector with a Merlin Microseal septum system and a 0.75-mm i.d. glass liner was used for thermal desorption of the SPME fiber. The injector temperature was 250 °C and the split valve was closed for 2 min. Injector pressure was 31.37 psi with a constant flow (0.9 ml/min) of helium carrier gas. The oven temperature program was started at 40 °C, held for 2 min and then programmed at 10 °C/min to 300 °C and held for 2 min. A single quadrupole mass spectrometer was used for detection in EI (+70 eV) mode. The source temperature was set to 230 °C, the analyzer to 150 °C, and the transfer line temperature to 280 °C. An m/z = 45–350 mass range was scanned at five full scans per second for the fiber selection procedure. Selected ion monitoring (SIM) was performed for calibration curves and for stent quantitation. The following ions were selected for SIM; m/z = 56 for lactide (data not shown), m/z = 105 and 122 for benzoic acid and m/z = 105 and 106 for benzaldehyde.

2.4. SPME extraction procedure

Manual SPME sample extraction was performed at 85 °C for 30 min directly from sealed vials for the optimization studies. The samples were equilibrated for 60 min at 85 °C prior to extraction. Automated SPME extraction was performed for the analysis of the samples using a Gerstel MPS-2 sampler. Using the Gerstel multipurpose sampler, the samples were equilibrated for 30 min at 85 °C and extracted for 30 min while being agitated at 250 rpm.

2.5. Optimization of the extraction procedure

The sample extraction and incubation times were optimized to the benzoic acid peak. Vials were pre-treated by covering the inner surface of the vial with a 75/25 PLGA polymer film. A solution of PLGA in acetonitrile (1 mg PLGA) was placed in the vials and allowed to dry. After drying of the film, the film was spiked with 50 ng of benzoic acid in aqueous solution (50 mg/L concentration), and extracted with a PDMS/DVB extraction fiber. Various equilibration times were evaluated between 10 and 50 min with 15 min of extraction time. The data shows that the peak areas did not change significantly after 30 min of equilibrium. Various extraction times were tested between 5 and 30 min with 30 min of equilibration. The data shows that there were no significant area changes after 15 min of extraction time. Based on this optimization work, an extraction time of 30 min and an equilibration time of 30 min were chosen. The agitation speed and the extraction temperature were not optimized in this study, however the same conditions were used that were previously optimized for polymer matrices in the authors laboratory.

2.6. Calibration curves

Standard curves for benzaldehyde and benzoic acid were constructed by extracting known amounts of standards spiked onto a PLGA film. Vials containing standards were extracted by automated SPME and the peaks were detected in SIM mode (monitored

ions described in the GC–MS conditions section). Extracted ion chromatograms $m/z = 122$ for benzoic acid and $m/z = 105$ for benzaldehyde were used to prepare the calibration curves. Calibration solutions were prepared by using a solution of individual analytes in aqueous solutions covering the range of 0.1–2500 ng/μL. The standard solutions (1 μL) were injected into pre-treated vials. The vials were pre-treated covered with 1 mg of 75/25 PLGA polymer film to simulate the stent matrix. The polymer was dried prior to application of the calibration solution. Triplicate preparations of each component were prepared at each level. Benzaldehyde responded linearly from 2 to 500 ng and the detection limit was 0.5 ng ($y = 24189x - 1532$, $R^2 = 0.998$), benzoic acid responded linearly from 5 to 2500 ng and the detection limit was 0.5 ng ($y = 69802x - 1782$, $R^2 = 0.999$).

3. Results and discussion

3.1. Fiber selection

The extraction efficiency of the previously mentioned SPME fibers were tested to compare the performance in extracting benzaldehyde and benzoic acid from paclitaxel eluting stents. For comparison of the fiber extraction efficiency, e-beam sterilized stents at 75 kGy were extracted manually using the various fibers. The SPME extraction fibers were analyzed by GC–MS and the extracted components were detected in scan mode. Fig. 1 shows the comparison of the different extraction fibers. The comparison is based on the extracted ion chromatogram of ion $m/z = 105$.

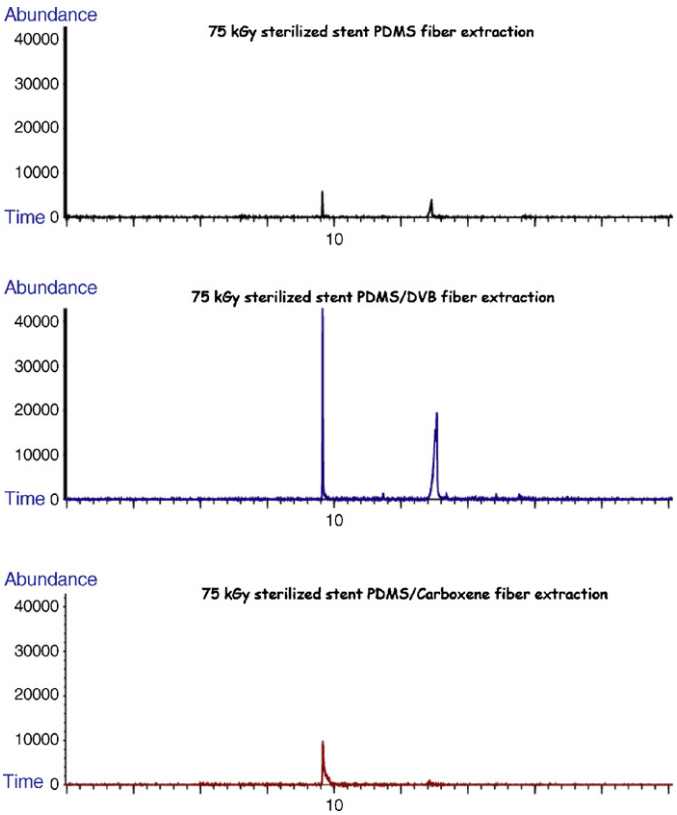

Fig. 1. Ion chromatogram of $m/z = 105$ ion, benzaldehyde (9.65 min) and the benzoic acid (12.9 min). 3× e-beam sterilized stents were extracted using a 65-μm PDMS/DVB extraction fiber (middle trace), a 100-μm PDMS extraction fiber (upper trace) and a 75-μm Carboxene/PDMS fiber (bottom trace).

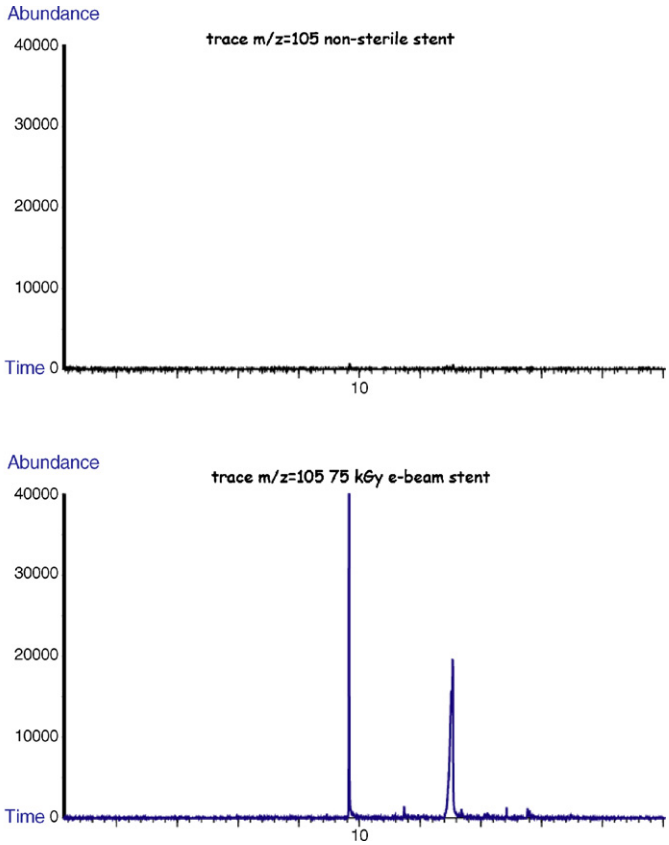

Fig. 2. Ion chromatogram of $m/z = 105$ ion, benzaldehyde (9.65 min) and the benzoic acid (12.9 min). Control (non-sterile) vs. 3× e-beam sterilized stents were extracted using a 65-μm PDMS/DVB extraction fiber. The upper trace shows the $m/z = 105$ extracted ion chromatogram of non-sterile stent, the lower trace shows the same extracted ion chromatogram for a 75-kGy e-beam sterilized stent.

As is shown in Fig. 1, the most efficient extraction fiber is the PDMS/DVB fiber, for both benzaldehyde and benzoic acid. Based on the extracted peak areas the PDMS/DVB extraction fiber is most effective for extracting these polar aromatic compounds. The observed result shows a good correlation with literature data [15]. The peak shape is far from ideal, and the "shark-fin" peak shape indicates a stationary phase incompatibility with the analyzed component. (The polar analytes have solubility issue with the apolar stationary phase, however due to the GC–MS analysis, polar columns such as Carbowax or cyanopropyl cannot be used because of excessive bleeding.)

3.2. Analysis of samples

The stent samples were extracted and processed the same way as the standards described above, except the vials were not pretreated since the stents already contain the polymer. Five individual non-sterilized stents, five stents sterilized at 25 kGy, and four stents sterilized at 75 kGy were analyzed.

Fig. 2 shows a representative extracted ion trace of $m/z = 105$ for a non-sterile vs. sterile stent. As is observed from the chromatogram in Fig. 2, the benzaldehyde and benzoic acid peaks show a significant elevation in the stents sterilized at 75 kGy. The concentrations of benzoic acid and benzaldehyde in the stents were calculated from the prepared calibration curves.

The results are shown in Table 1. The non-sterile stents do not contain benzaldehyde or benzoic acid above the detection limit. The stents sterilized at 25 kGy contain elevated levels of ben-

zaldehyde. In the 75 kGy sterilized stents the benzaldehyde level is not significantly higher than in the 25 kGy stents. The lack of increase in benzaldehyde indicates that it may be an intermediary to benzoic acid formation due to the further oxidation of the molecule to benzoic acid. Benzoic acid level is more than 50× higher in the 75 kGy samples relative to the control samples. The proposed mechanism of formation for benzoic acid and benzaldehyde from paclitaxel is summarized in Fig. 3. As reported in the literature paclitaxel is relatively easily degrading to baccatin III and the methyl-ester side chain counterpart [2,14]. Baccatin III is the circled portion of the molecule in Fig. 3. The dotted lines in the paclitaxel structure in Fig. 3 indicate the sites for loss of phenylacylium radicals, which are known to form under irradiation conditions [17]. The phenylacylium radical can extract hydrogen from the acidic PLGA matrix resulting in benzaldehyde (Fig. 3/a) or the radical can also be quenched by a hydroxyl radical from residual moisture forming benzoic acid (Fig. 3/b). Benzoic acid can also arise from the resulting benzaldehyde by a two-step process. First the hydroperoxide intermediate is formed, which is reduced to generate benzoic acid, concurrently DMSO (filling solvent) undergoes oxidation to form dimethyl-sulfone, another observed side product in this system (Fig. 3/c). Additionally, paclitaxel degradation could occur at the locations indicated by solid lines in Fig. 3 [17], directly forming benzoic acid or benzamide by extraction of

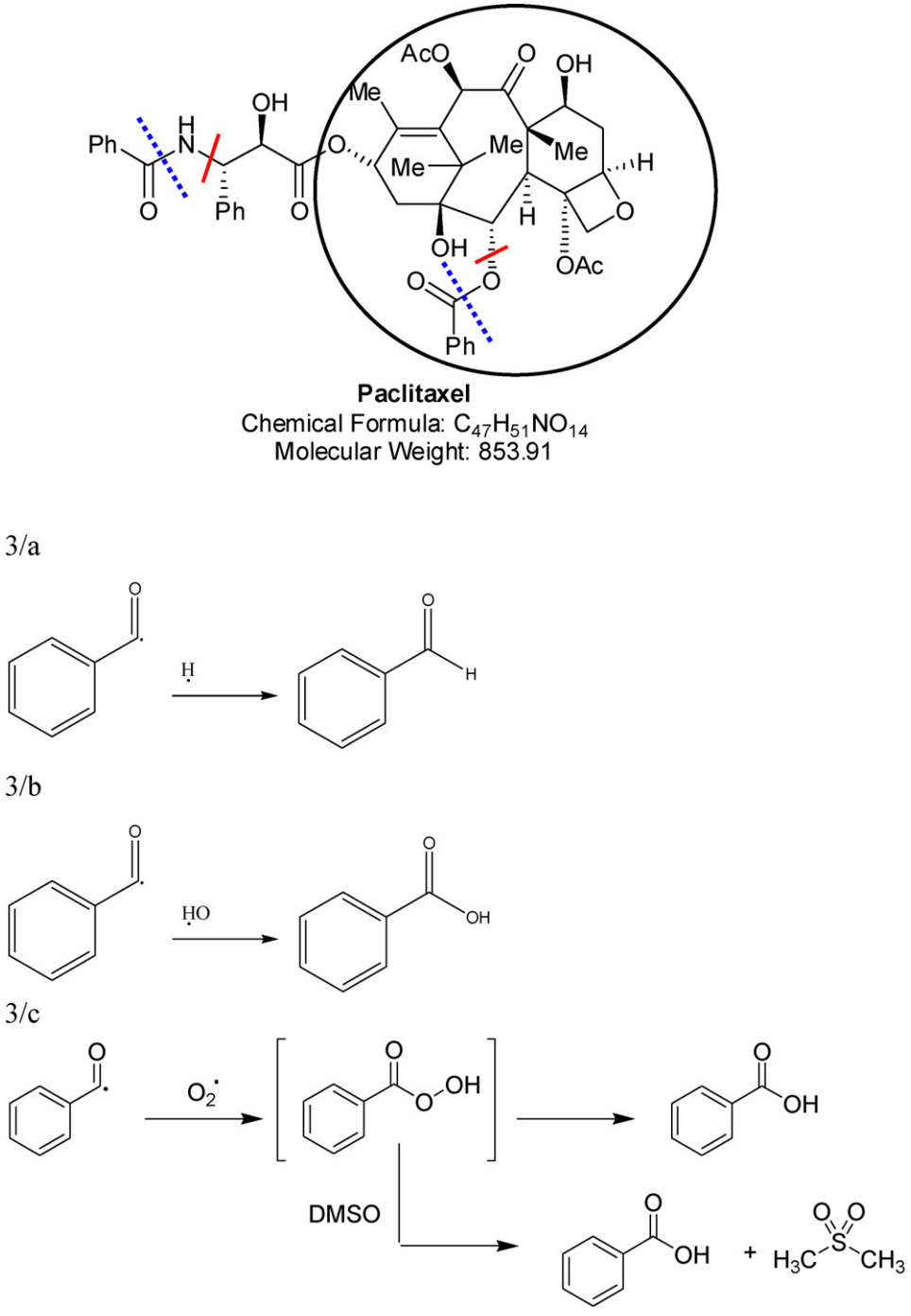

Fig. 3. Chemical structure and proposed degradation pathways of the paclitaxel. The lines shows a possible elimination sites of volatile compounds.

Table 1
Calculated analyte values for non-sterile, 25 and 75 kGy e-beam sterilized stents

Stent	Amount (ng)	
	Benzaldehyde	Benzoic acid
Non-sterile		
0_1	ND	ND
0_2	ND	ND
0_3	ND	ND
0_4	ND	ND
0_5	ND	ND
Average	NA	NA
1× sterile		
1_1	9.4	3.7[a]
1_2	4.6	7.6[a]
1_3	4.7	2.6[a]
1_4	4.3	2.5[a]
1_5	4.5	1.4[a]
Average	5.5	3.5[a]
3× sterile		
3_1	6.0	232.6
3_2	8.1	232.9
3_3	5.9	271.7
3_4	5.3	441.7
Average	6.3	294.7

[a] Estimated levels are above the detection limit but below the linear range of the calibration curve (ND, not detected and NA, not applicable).

a hydrogen radical from the PLGA matrix. Benzamide was tentatively identified, based on the GC–MS electron impact spectra, and detected at very low levels in the sterilized sample indicating that these cleavages are unfavorable. The data in Table 1 shows that the e-beam does have an impact on the formation of volatile paclitaxel related compounds. There is some variation in the levels of benzoic acid and benzaldehyde observed on the stents sterilized at 25 and 75 kGy. The varying levels of benzaldehyde and benzoic acid may be due to typical variations in the manufactured stents and localized heating effects. The levels of benzoic acid and benzaldehyde observed on the stents did not resolve the original mass-balance issue, but most likely contribute to the lack of mass balance observed for paclitaxel on the CoStar stents.

4. Conclusion

The loss of volatile compounds contributed to the lack of mass balance of paclitaxel. Volatile paclitaxel degradants, benzoic acid and benzaldehyde, were found on the e-beam sterilized coronary stents. Benzaldehyde and benzoic acid were extracted from the paclitaxel eluting stents using a relatively simple SPME-GC–MS method. The optimum SPME extraction was obtained with a 65-μm PDMS/DVB extraction fiber, which was subsequently analyzed by GC–MS in SIM mode. The detection limits for benzaldehyde and benzoic acid were determined to be 0.5 ng. The e-beam dose affected the levels of benzoic acid and benzaldehyde found on the stents. The controls contained levels below the detection limit for benzoic acid and benzaldehyde, while the 25 kGy e-beam sterilization increased the level of benzoic acid significantly relative to the control. The 75 kGy e-beam sterilization increased the benzoic acid level significantly relative to the 25 kGy e-beam sterilization. The levels of benzaldehyde increased in the sterilized stents relative to the control stents, however there was no change between the 25 kGy dose and the 75 kGy dose. A mechanism for the loss and formation of benzoic acid and benzaldehyde was proposed.

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