Development of FTIR and HPLC Methods for Analysis of Substances Extracted from Hydrophilic Urinary Catheters

*Master of Science Thesis in Chemical and Biological Engineering, KBT X05*

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Göteborg, Sweden, 2011
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Cover:
Extraction of urinary catheters in water and isopropyl alcohol (see 3.3.1)

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Abstract

On request from Astra Tech, this M.Sc. Thesis is about method development and analysis of extracted urinary catheters with both FTIR and HPLC methods. The aim of these methods is to be able to analyze what substances a patient may be exposed to when using the product.

Two HPLC-methods with different columns were developed to investigate which column had the best separation and shortest retention time. The methods were created for analysis for a mixture of standard additives of interest that are thought to be present and can be extracted from the catheters. From previously developed methods, literature searching and from trial and errors the additives were separated and the methods were optimized by changes of eluent composition, column temperature, flow rates and type of columns.

The main additives of interest were Irganox 1010, Irgafos 168 and DEHP. The HPLC-methods were then used to analyze extracts from five catheters manufactured from different raw materials, each exposed to different radiation doses and with or without PVP-coating. The extracting mediums were isopropyl alcohol and Milli-Q water in which pieces of catheters were placed in 70 °C for 24 hours.

Both the solid catheters and extracts of catheters were also analyzed using FTIR. This technique was used to get a fingerprint of what the catheters consist of and what might be extracted from them. The FTIR-spectra were compared with both on site made spectra of additives and spectra from literature.

In both the FTIR-spectra and the HPLC-chromatograms, it could be seen that the extraction solutions from catheters extracted in Milli-Q water did not contain any additives or very small amounts of it. Additives from the catheters in isopropyl alcohol however, were extracted in detectable concentrations. Differences between coated and uncoated catheters could easily be seen with both FTIR and HPLC and the HPLC analysis also showed differences in concentration due to radiation doses.
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1 Abbreviations

The different abbreviations used in the report are as follows:

ACN ........................ Acetonitrile
BHT ........................ Butylated Hydroxytoluene
CAS ........................ Chemical Abstracts Service
DEHP ........................ Di (2-ethylhexyl) phthalate
DNA ........................ Deoxyribonucleic acid
FTIR ........................ Fourier transform Infrared spectroscopy
HPLC ........................ High performance liquid chromatography
IPA ........................ Isopropyl alcohol
MQ-water .................... Milli-Q water
PA ........................ Polyamide
PE ........................ Polyethene
PEBA ........................ Poly (ether-block-amide)
POBE ........................ Polyolefin based elastomer
PVC ........................ Polyvinyl chloride
PVP ........................ Polyvinylpyrrolidone
SBC ........................ Styrenic block copolymers
UATR ........................ Universal Attenuated Total Reflectance
UV ........................ Ultraviolet
1,3-DTBB ........................ 1,3-di-tert-butylbenzene
2,4-DTBP ........................ 2,4-di-tert-butylphenol
2 Introduction

Astra Tech AB is a subsidiary of Astra Zeneca and develops, produces and markets dental implants and advanced healthcare products within urology and surgery. This M.Sc. Thesis was conducted within the Urology R&D section at Astra Tech, which develops products and packaging within the urology area. The main product is LoFric, a hydrophilic, single usage, urinary catheter and is used by patients who do not have proper control of their bladder functions. [1]

The catheter consists of a plastic tube with a hydrophilic polymer coating. This coating mostly consists of Polyvinylpyrrolidone, PVP, and will absorb and hold water when wet. By soaking the PVP layer before use, friction between the catheter and the urethra can be minimized, reducing the risk of damage. The catheters are sterilized by electron beam irradiation. This sterilization may affect the hydrophilic coating as well as the raw material of the catheter so that degradation products are formed. [1]

Different legislations, directives and control organs require that the manufacturer of medical products have a declaration of which compounds the user could be exposed to. This requires relevant methods for analyzing and quantifying these compounds. For these reasons there is a need for and interest in determining which substances that can be extracted from the LoFric catheter to the urethra and urinary bladder during catheterization. [1]

Today, Astra Tech AB has a great interest in developing appropriate methods for identification of substances extracting from both the catheter material and the catheter coating.

The focus of this M.Sc. Thesis was to develop a new liquid chromatographic method for analyzing extracted substances from urinary catheters. Fourier transform Infrared spectroscopy, FTIR, was also used to get a fingerprint of the composition of the catheter as well as the extracts of the catheter.
3 Theory

A short description about the catheters, the catheter material and the techniques used is given in this chapter.

3.1 The urinary catheter

The ability to retain urine and to empty the bladder can be affected in a number of ways, for example by sickness, ageing or by an injury. These patients without proper control of their bladder function can therefore use a urinary catheter for emptying of the bladder.

3.1.1 Construction and function

Astra Tech has a wide range of different catheters for different needs. The standard catheters called LoFric consist of four different parts (see figure 1). There is different length on the catheters and the color on the connector symbolizes the size, or Charrière (CH), for the catheter. The Charrière unit is equivalent with one millimeter and symbolizes the catheter-pipe diameter. The emptying with these catheters is called CIC, which stands for Clean Intermittent Catheterization, which is a sterile regular technique for emptying of the bladder by catheterization. Before usage, the catheters are soaked in water for 30 seconds to absorb water to minimize friction in the urethra. [1]

![Figure 1. Construction of the catheter.](image)

1. Connector
2. Catheter
3. Opening, for the urine to drain
4. Catheter tip

3.1.2 Catheter coating

The catheters are covered with a hydrophilic coating to minimize friction at usage. The coating consists of PVP and sodium chloride, NaCl, which gives, when wet, a slippery coating that reduces the friction by 90-95 % compared with catheters using gel (see figure 2). The salt concentration in the catheter coating is the same as the salt concentration in the urethra which makes the coating isotonic. This prevents the water from diffusing out of the hydrophilic coating and results in a catheter that is slippery during the whole catheterization process. This is called the “Urotonic Surface Technology” and is a patented coating. [1]
3.2 Materials

The catheters consist of different materials but also different additives are added to the material to obtain different properties. The materials and the most common additives are described below.

3.2.1 Polyvinyl chloride - PVC

After polyethylene, polyvinyl chloride, PVC, is the most widespread basic plastic in the world. PVC is a thermoplastic, solid material in room temperature, and is produced from NaCl and ethylene. Ethylene is chlorinated to dichloroethane and vinyl chloride monomers that are polymerized to PVC (see figure 3). [2]

![Figure 3. Polyvinyl chloride – PVC.](image)

3.2.2 Polyolefin based elastomer - POBE

POBE is a copolymer and consists of polypropylene or polyethylene and an alpha-polyolefin such as polybuthene or polyoctene (see figure 4). Polyolefins are some of the major commercial thermoplastics and are synthesized from simple olefins, so-called olefinic monomers or in other words alkenes. The crystallinity in polyethylene or polypropylene is disturbed by the incorporation of comonomers which results in an increasing elasticity with increasing incorporation. POBE has the ability to be cross-linked during irradiation and is used in rubber and plastic applications. [3, 4, 5]

![Figure 4. Possible building blocks for a POBE material.](image)
3.2.3 **Styrenic block copolymers - SBC**

Styrene-Butadiene-Styrene copolymer, SBS, and Styrene-Ethylene-Butadiene-Styrene, SEBS, see figure 5 and 6, are both styrenic block copolymers, SBC’s, and are based on simple molecules of the type \( \text{A–B–A} \). It forms two-phase systems which separately retain many of the homopolymer properties which result in two glass transition temperatures, \( T_g \). Because of this, in the SBC’s at room temperature, the polystyrene is rigid and strong and the elastomer is elastic and can be extended. The middle section is the difference between SBS and SEBS and it is this completely hydrated middle section that gives SEBS a great resistance against weathering, ozone- and UV exposure. [6, 7]

![Figure 5. Styrene-Butadiene-Styrene – SBS.](image)

![Figure 6. Styrene-Ethylene-Butadiene-Styrene – SEBS.](image)

3.2.4 **Poly (ether-block-amide) - PEBA**

Polyether-block-amide, PEBA, belongs to the group of segmented block copolymers. The soft segment is based on aliphatic polyether and the hard segment is based on aliphatic polyamides and the segments are linked by amide groups, see figure 7. The aliphatic polyether segment is flexible and extended and the aliphatic polyamide segment acts as a physical cross-link that reduces the slippage of the chain and viscous flow from the copolymer. [6]

![Figure 7. Poly (ether-block-amide) – PEBA.](image)
PEBA is produced from carboxylic acid-terminated aliphatic amide blocks and hydroxyl-terminated polyether diols through esterification, see figure 8. [6]

\[
\text{HO-PA-COOH} + \text{HO-}[\text{C}_2\text{H}_4\text{n}]\text{OH} \rightarrow \text{HO-}[\text{PA-COOE O PE-}]_n\text{H}
\]

Figure 8. Synthesis of PEBA.

### 3.2.5 Polyvinylpyrrolidone – PVP

Polyvinylpyrrolidone or PVP, see figure 9, is a water-soluble polymer and has been used in a wide range of applications, for example as a blood plasma substitute. It is also soluble in a wide range of materials, for example halogenated hydrocarbons and many alcohols. [8]

Vinylpyrrolidone is synthesized from acetylene, formaldehyde and ammonia. PVP are then processed in a water solution. This gives a solution that contains 30 % PVP. By using of a spray it can then be dried which gives a fine powder. [8]

Figure 9. Polyvinylpyrrolidone – PVP.

### 3.2.6 Plastic additives

To get specific properties in a plastic material, different additives are added. Common types of additives in plastics are, for example:

**Fillers** – By weight, fillers are the most common additives. They are mostly used as a price reduction agent but they can also improve the toughness, strength, stiffness or hardness. The most widely used filler in polymers are calcium carbonate. [9, 10]

**Plasticizers** – The main use of plasticizers is to give softness and flexibility. They are often di- or tri-esters of anhydrides and aromatic or aliphatic acids. A commonly used plasticizer is Di (2-ethylhexyl) phthalate, DEHP. [9, 10]

**Flame Retardants** – Flame retardants are used to provide flame and fire resistance and to prevent ignition or spread of flame in the material. Commonly used are brominated hydrocarbons. [9, 10]
**Stabilizers** – There are different types of stabilizers, for example heat or light stabilizers. Heat stabilizers are used to prevent thermal decomposition of the polymer and the light stabilizers are used to prevent decomposition, discoloration or embrittlement due to UV-light. Organometallic compounds such as lead or tin compounds are commonly used as heat stabilizers. [9, 10]

**Colorants/Pigments** – Pigments are used to give the plastic a specific color and are often also light stabilizers. There are two different types: organic - dyes, and inorganic - pigments. The most common pigment for outdoor use is titanium dioxide. [9, 10]

**Lubricants** – The main use for lubricants are to improve the flow characteristics, reduce the friction and prevent plastic melt from sticking to the molds during the processing. There are two different types of lubricants, external and internal. External are used for coating of the equipment to reduce friction, delay fusion and give melt control. They do not interact with the polymer itself. The internal lubricants reduce the friction between the polymers and are chemically compatible with the polymer. They also lower the melt viscosity by reducing the van der Waals forces. Most commonly used are metallic stearates. [9, 10]

**Antioxidants** – Antioxidants are used to prevent degradation due to oxidation. [9, 10]

### 3.2.6.1 Common additives

Some common additives, studied in this thesis, are listed below.

#### 3.2.6.1.1 Di (2-ethylhexyl) phthalate – DEHP

Di (2-ethylhexyl) phthalate (see figure 10) is a plasticizer for PVC and is produced of phthalic acid anhydride and 2-ethylhexanol during esterification under high temperature. It is a colorless and viscous liquid with low vapor pressure and is insoluble in water. Because of its insolubility in water, DEHP has a very slow leaching rate into landfill. [11]

![Figure 10. DEHP, CAS: 117-81-7](image-url)
3.2.6.1.2 Tetrakis (3-(3,5-di-tert-butyl-4-hydroxyphenyl)propionate) - Irganox 1010

Irganox 1010 (see figure 11) is used as a stabilizer/antioxidant for polymeric and organic materials. It is non-discoloring and prevents thermo-oxidative degradation. [12]

![Irganox 1010, CAS: 6683-19-8](image)

**Figure 11.** Irganox 1010, CAS: 6683-19-8

3.2.6.1.3 Tris (2,4-di-tert-butylphenyl) phosphite - Irgafos 168

Irgafos 168 (see figure 12) is used in polymers as a non-discoloring antioxidant. This additive easily reacts to form its oxidation product, tris(2,4-di-tert-butylphenol)phosphate (figure 15) but it can also be hydrolyzed into 2,4-di-tert-butylphenol (figure 13) or if subjected to radiation, can decays into 1,3-di-tert-butylbenzene (figure 14). [12, 13, 14]

![Irgafos 168 - Tris (2,4-di-tert-butylphenyl) phosphite](image)

**Figure 12.** Irgafos 168 - Tris (2,4-di-tert-butylphenyl) phosphite
3.2.6.1.4 Butylated Hydroxytoluene – BHT

BHT is used as an antioxidant in for example plastics and rubber (see figure 16). It is a white crystalline solid that is insoluble in water but easily soluble in IPA. It is also used as an antioxidant in foods. [15]

3.2.6.1.5 Erucamide

Erucamide is an aliphatic amide and are used as a foam stabilizer and antiblock agent for polyethylene (see figure 17). Erucamide is soluble in IPA but insoluble in water. [15]

3.3 Techniques

Brief descriptions of the techniques used are listed below.

3.3.1 Extraction

The catheters were cut a number of times across the catheter and once along with the catheter (see figure 18). This was done to avoid air bubbles inside the catheter affecting the area contacting the solvent. The pieces were then placed in 10 ml of solvent in a closed vessel. The used solvents were Milli-Q water, MQ-water, and isopropyl alcohol, IPA.
These vessels were placed in a furnace at 70 °C for 24 hours. According to the ISO 10993 standard, the amount of sample should be 3-6 cm² catheter surface / ml solvent.

The reason for using IPA and water as solvents derives from the standard, ISO 10993, which requires that a medical device / product has to be extracted in both a non-polar, medium-polar and polar solvent. Normally used solvents are water, IPA and hexane. Hexane is not tested in this thesis.

**Figure 18.** How the catheter was cut.

Equation for deciding the length of the catheter placed in the solvent:

\[
\begin{align*}
\text{Outer radius}: & \quad R_{\text{CH12}} = 0.20 \text{ cm} \\
& \quad R_{\text{CH14}} = 0.23 \text{ cm} \\
\text{Inner radius}: & \quad r_{\text{CH12}} = 0.13 \text{ cm} \\
& \quad r_{\text{CH14}} = 0.16 \text{ cm} \\
\text{Outer area} : & \quad A_{\text{outer}} = 2\pi Rh \\
\text{Inner area} : & \quad A_{\text{inner}} = 2\pi rh \\
\text{Area of cut surface} : & \quad A_{\text{cutted}} = 2X(2\pi R^2 - 2\pi r^2) + 4h(R - r) \\
\text{Total area} : & \quad A_{\text{tot}} = 2\pi h(R + r) + 2X(2\pi R^2 - 2\pi r^2) + 4h(R - r) \\
\text{Length of the catheter} : & \quad h = \frac{A_{\text{tot}} - 4X\pi(R^2 - r^2)}{2\pi(R + r) + 4(R - r)}
\end{align*}
\]

Where X is the number of lengthwise pieces of the catheter

**Table 1.** These values are been used in all the extractions:

<table>
<thead>
<tr>
<th>Catheter size</th>
<th>( A_{\text{tot}} ) (cm²)</th>
<th>X (bits)</th>
<th>h (cm)</th>
<th>( V_{\text{extracting medium}} ) (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH12</td>
<td>50</td>
<td>20</td>
<td>18,8</td>
<td>10</td>
</tr>
<tr>
<td>CH14</td>
<td>50</td>
<td>20</td>
<td>16,8</td>
<td>10</td>
</tr>
</tbody>
</table>
3.3.2 Sterilization

Before use, the catheters are sterilized by irradiation or ethylene oxide.

3.3.2.1 Electron beam irradiation – E-beam

The main sterilization technique used at Astra Tech is electron beam irradiation, which is often used in industries. Electron beam irradiation works by bombarding the target with high energy electrons. The interaction between the electrons and the generated radicals results in a DNA chain cleavage in living organisms, for example bacteria, and results in microbial death, leaving the irradiated sample sterile (see figure 19). [16, 17]

Some benefits with using this technique are that the sterilization is quick and simple so that it is easy to use in an industrial scale. The process uses no radioactive source and the products are only exposed for the radiation for a few minutes. Some disadvantages are that it may affect the physical properties of the thermoplastic product, such as its color. The penetration capability is also poor, only around 5 cm, so the product may need to be sterilized many times from different sides. [16]

The standard sterilization used in Astra Tech is beta irradiation at 56 kGy.

![Figure 19. Degradation of the DNA chain due to E-beam irradiation.](image)

3.3.2.2 Ethylene oxide

Previously, ethylene oxide treatment was used as sterilization process but now it has been replaced with electron beam irradiation. This is due to that ethylene oxide does not work on solvents and the plastic covers around the catheters must have an opening for the gas to enter. This can either be done by an actual opening in the cover or by a special paper window in the bag. The process for this is more complicated and together with the fact that ethylene oxide is a toxic gas with restriction the E-beam sterilization is the main technique used today. [18]
3.3.3 Fourier transform Infrared spectroscopy - FTIR

Infrared spectroscopy is a very important technique in qualitative analysis. A compound that has a covalent bond and a dipole moment can absorb frequencies of electromagnetic radiation in the infrared region. To absorb energy, the bond must have a dipole moment that changes at the same frequency as the incoming radiation. Different molecules absorb at different energies/frequencies and thus have different absorption patterns. The absorbed energy increases the amplitude of the vibrational motion, exciting the molecule to a higher energetic state. There are two common types of spectrophotometers, dispersive and Fourier transform (FT) instruments. Only the FTIR will be used in this thesis. [19, 20]

The FTIR instrument, see figure 20, uses an interferometer for generation of a spectrum. A chopper or beam splitter divides the radiation from the infrared source in two equal parts. Half of the light hits a fixed mirror and half of it hits a mirror that is moving at high speed. The beams will be out of phase to each other when they meet again. They will then be recombined and the result will be an interference pattern where a specific wavelength is reinforced at a specific time depending on the position of the moving mirror. The radiation that goes through the sample will contain light of all wavelengths but because of the reinforcing of wavelengths, depending on the moving mirror, the detection signal can be transformed to a spectrum where transmittance or absorbance are plotted against the wavelength. This is done using Fourier transform. [19]

![Figure 20. Fourier transform infrared spectrometer][19]

Benefits for FTIR as compared to a dispersive instrument are that a spectrum can be generated in a very short time, sometimes less than a second, and a mean can be calculated during series of measurements that improves the signal-to-noise ratio. [19]
The IR-spectrum will be used as fingerprints for the different catheters and extraction media.

Figure 21. Analysis with FT-IR.

There are different ways of analyzing with FT-IR, see figure 21. One way is using a cuvette when analyzing a liquid sample. Another example is when analyzing on solid samples or on liquids, a UATR – Universal Attenuated Total Reflectance, see figure 22, can be used instead of a cuvette. Solid samples are held in place by an arm that applies a pressure on the sample to get a good connection between the sample and the crystal. For liquid samples, a drop of the sample is placed on the crystal. [21]

Figure 22. UATR Crystal.

Samples in volatile solvents can be analyzed with Crystal IR sample cards. A drop of the sample are placed on the window, the solvent will then transpire which yields a spectrum without peaks from the solvent. Another benefit is that no harmful, volatile solvent are used outside a fume hood or on the equipment. [21]

3.3.4 High performance liquid chromatography - HPLC

High performance liquid chromatography, or HPLC, is the most common liquid chromatographic technique for separation of analytes in complex mixtures. The molecules, dissolved in a mobile phase, are passed through a column and are there separated. The column consists of tightly packed solid particles, a so-called stationary phase, and the reason for the separation of the molecules is that the molecules interact differently with the stationary phase. The molecules with stronger interaction with the stationary phase pass slowly through the column, while molecules with weaker interaction pass more rapidly. Retention time is the time it takes for a molecule to pass through the column and to the detector. For a schematic description of a HPLC-system, see figure 23. [22, 23]
The stationary phase can consist of two different phase types, straight phase or reversed phase:

3.3.4.1 Straight phase HPLC

Straight phase uses a polar stationary phase and either a non-polar mobile phase or weakly polar solvent where the analytes are dissolved. The column is filled with silica particles and the separation of molecules is due to polarity or adsorption, interaction strength, but also steric factors. A polar analyte will interact more strongly with the polar silica than a non-polar analyte. This generates different retention times where the non-polar molecules will pass through the column first and the rest will pass in order of increased polarity. [22, 23]

3.3.4.2 Reversed phase HPLC

Reversed phase uses non polar stationary phases and are the most commonly used HPLC method. This phase consists of modified silica with non polar alkyl chains on the surface. The most common is alkyl chains with 18 carbons which are called C\textsubscript{18}-phases. The mobile phase is often a polar water mixture. Polar molecules in the solvent will therefore be moved with the mobile phase due to of the strong attraction between the polar solvent and the polar molecules and also because of the lack of attraction or low attraction to the alkyl chain on the silica in the stationary phase. Because of van der Waals forces to the alkyl chain and because of less solubility in the solvent the non polar molecules will move more slowly through the column. Summarized, the most polar molecules are the first to pass through the column and the other molecules will then follow with decreasing polarity. An overview of a chromatogram from reversed and straight phase HPLC can be seen in figure 24. [22, 23]
The result is a series of peaks where each peak represents a compound that have passed through the column and the detector and absorbed UV light. The retention time for the molecules can be used to identify the compounds or the peaks can be used for quantification of the compounds. The area under a peak is proportional to the amount of compound. Caution shall be taken if two or more compounds are analyzed. The different compounds may absorb UV light more or less at a certain wavelength which will result in different areas under the peaks, but it does not mean that one compound is present in a larger quantity than the other. [23]

3.3.4.3 Method development for HPLC

There are several steps and ways to develop and optimize an HPLC method. The aim is to get a satisfactory separation of the ingoing components in a sample within a reasonable time interval. The method should also be stable and reliable for small changes. [25, 26] Some of the main steps are as follows:

1. **Consult literature, define a method and understand the chemistry.**

   Determine the goal of the method. Searching in literature for other, previously used methods for the separation and if it has been done before and if so, under which conditions. If the separation has not been done before, determine the aim of the method development and define a HPLC method that will be able to analyze the sample. [25, 26]

   To consider:
   - Reversed or straight phase
   - Mobile phases
   - Gradient or isocratic flow
   - Column - length, size, flow rate, packing particle size
   - Detector

![Figure 24. Straight phase and revered phase [24]](image)
2. **Initial conditions.**
   Determine the optimum condition for selected properties in step one. The optimum is an acceptable separation at a minimal run time. [25, 26] This can be done by changing the following properties:
   - Mobile phases – change the strength of the mobile phases and find the optimal concentration for the strong solvent.
   - Gradient flow can be used if the sample contains a large number of analytes or if the retention time is long.

3. **Sample preparation**
   Define a sample preparation by determining which solvent that should be used, extraction volume and sample concentration. [25, 26]

4. **Optimization**
   Find a satisfactory balance between the retention time and the separation. Find the weaknesses and optimize the method. This is done through experimental design. [25, 26]

5. **Method validation**
   To verify the method and to test it for the purpose it should be used for, several tests must be done. [25, 26]

3.3.4.4 Possible separation problems with reversed phase HPLC

At an initial gradient separation, a series of separation problems may occur. It is important to correct these problems early thus later the correction may affect the separation and all the work for the method can be in vain.

3.3.4.4.1 Late elution time
Molecules that interact strongly with the stationary phase inside the column have a longer elution time than molecules with weaker or no interaction. To accelerate the elution time, the composition of the mobile phase can be changed or a gradient of the mobile phase from water to Acetonitrile, ACN, can be used. If the sample are too non-polar, the gradient of the mobile phase, water/ACN can be replaced by a gradient from ACN to a less polar solvent. A change in the flow rate can also minimize the elution time. [27]

3.3.4.4.2 Early elution time
Molecules that interact weakly or not at all with the stationary phase will elute very early. Examples are small, polar molecules.
One way to extend the elution time is to start a gradient with a lower concentration of ACN in the mobile phase. A decrease in the pH will decrease the ionization for acids and increase it for bases and vice versa. [27]

3.3.4.4.3 Peak broadening

Molecules from the injected sample are, in the beginning, in a small volume with a constant concentration. During analysis, these molecules are distributed in a larger volume. Separation problems can then occur when two adjacent peaks overlap. The separation will then be incomplete. Peak broadening can not be avoided but different settings can minimize it. Reasons for peak broadening are for example, the diffusion in the mobile or stationary phase, slow kinetics, different flow rates during the analysis or different molecules have to pass different ways through the column. [24]

3.3.4.4.4 Peak tailing

It is not unusual that peaks in a spectrum is tailing. Causes of tailing peaks can be contamination in the column, poorly constructed column, the injection volume is too large, the sample is injected in an inappropriate way or the column is not suitable for the analyzed samples. With tailing peaks it is difficult to reach an optimal separation and difficulty to implement quantitative analysis. A measurement of peak tailing is the tailing factor, \( T_f \). See also figure 25. [24, 28]

\[
T_f = \frac{W_{0.05}}{2a} = \frac{a + b}{2a}
\]

(Equation 6)

a and b are measured at h/20 where h is the peak height.

\( T_f < 1.0 \) Pre-tailing (uncommon)

\( T_f = 1.0 \) Good symmetry

\( T_f > 1.0 \) Tailing

\( T_f > 2.0 \) Unacceptable tailing

**Figure 25.** Peak tailing measurements
In ChemStation, the software for the HPLC-system used in this work, a more detailed peak symmetry calculation has been used and it is these peak calculations that are represented later in the report. In this calculation, more parameters are taken into account. A perfectly symmetrical peak is represented by a symmetry value of one.
4 Experimental

The experimental part was performed by FTIR analysis of solid catheter as well as on extracts and HPLC analysis of extracts. FTIR was used to get a fingerprint of different catheters and what possibly can be extracted out from the catheters into different solvents. This was then used to develop a method for identification of the substances in the catheter that can be extracted out in the analyzed solvent.

LoFric with five different catheter materials have been examined in this report (see appendix A:1):
- PVC - PVC material
- POBE I - POBE material
- POBE II - POBE material
- Mediprene - POBE material with SBS and SEBS as building blocks
- PEBAX - PEBA material

4.1 FTIR analysis

Four experiments were done in the FTIR part. Solid catheters were analyzed to get a reference of what can be extracted. A spectrum for DEHP was done for comparison of the PVC samples. Extraction of coated and uncoated catheters in IPA and MQ-water, were analyzed with FTIR. The analyzed catheters can be seen in appendix A:1 and the used equipment were:
- FT-IR Spectrum 100, SY-0270
  Range: 4000-400 cm⁻¹
  Scan number: 4 scan
- UATR with thallium bromide crystal (KRS-5)
- Furnace

4.1.1 Development of FTIR spectrum of additives and constituents from standards

Powder of PVP K30, PVP K90, Irganox 1010 and Irgafos 168 and a solution of IPA and 10 % (vol.) DEHP was analyzed with FTIR. This was done to give proper spectra for comparison with the analyzed samples and for determination of the absorbencies.
4.1.2 FTIR analysis on solid catheters

Five different uncoated catheters and two different coated catheters with different sterilization doses were analyzed with FTIR. First, they were analyzed without undergoing any sterilization process. They were then analyzed after being sterilized with beta radiation doses of 56 kGy, 100 kGy and 200 kGy. Four samples of the uncoated catheters and two samples of the coated catheters were made for each dose and for each catheter. These different samples were then examined against each other to see if there were any deviations between the spectra taken before and after the sterilization process. An interpretation for the different peaks was also done.

4.1.3 Extraction in MQ-water and FTIR analysis on uncoated and coated catheters

Five different uncoated catheters and two different coated catheters with sterilization dose 56 kGy, 100 kGy, 200 kGy and unsterilized were extracted in MQ-water. The extraction was done according to chapter 3.3.1. The extracts were analyzed with FTIR after 24 hours. The background for the FTIR spectra was done with pure MQ-water.

4.1.4 Extraction in IPA and FTIR analysis on uncoated and coated catheters

Five different uncoated and two different coated catheters with sterilization dose 56 kGy, 200 kGy and unsterilized were extracted in IPA. The extractions were done according to chapter 3.3.1. The extracts were analyzed with FTIR after 24 hours in 70 °C. The background measurement for the FTIR spectra was done with pure IPA.

4.2 HPLC analysis

For detection of the extracted additives, a method in HPLC was made and the separation of the peaks was optimized. A reference chromatogram from known additives was also made for detection of the peaks from the extracts of different catheters. This was done for both extraction solvents, MQ-water and IPA. All the extracts were analyzed after 24 hours in 70 °C.

The analyzed catheters can be seen in appendix A:1 and the used equipments are:

- LC-UV instrument, SY-0365
  
  Pre-column:
  
  Zorbax Eclipse XDB-C8
  
  2.1 x 12.5 mm
  
  3.5 µm
Column #1:
  Zorbax Eclipse Plus C18
  2.1 x 50 mm
  3.5 µm

Column #2:
  Zorbax Eclipse XDB-C8
  2.1 x 50 mm
  3.5 µm

Software: ChemStation

- HPLC filter, 30 nm, 0.45 µm, nylon membrane
- HPLC vial, 2 ml
- Mobile phase:
  - Eluent A: 10 % ACN
    90 % MQ-water
  - Eluent B: 100 % ACN

4.2.1 Method development for HPLC

The equipment and chemicals available for the method development is described above. From this, a method that separates peaks from different additives and PVP should be created.

From previous attempts and from literature studies, a first draft method was created. Because of major differences in retention time for different additives, a gradient was used.

Method settings:

<table>
<thead>
<tr>
<th>Injection volume:</th>
<th>3 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate:</td>
<td>0.3 ml/min</td>
</tr>
<tr>
<td>Column:</td>
<td>#1</td>
</tr>
<tr>
<td>Column temperature:</td>
<td>40.0 °C</td>
</tr>
<tr>
<td>Number of injections:</td>
<td>1</td>
</tr>
<tr>
<td>Eluent program:</td>
<td>See appendix C:5.1</td>
</tr>
</tbody>
</table>
The additives were diluted in IPA to proper concentrations to get approximately the same UV absorbance in the first draft method. The used additives and their concentrations were:

- Irganox 1010 in IPA, 0.05 mg/g
- Irgafos 168 in IPA, 0.11 mg/g
- DEHP in IPA, 0.24 mg/g
- 1,3-DTBB in IPA, 0.15 mg/g

IPA was used because of the acceptable solubility in IPA for all the additives.

During the method development, the additives were dissolved in the same vial since it is more like the extracted catheter samples and it is easier and faster to only run one sample at every change made during the development.

PVP K90 and PVP K30 were also tested, but they were eluted as the same time as the solvent (see appendix D:2). PVP was not of interest for detection, so no further runs were done for separating the solvent and the PVP peaks. An attempt to add 1,3-DTBB and BHT were also done but despite changes of the method 1,3-DTBB and BHT could not be separated.

In the beginning, signals at both 254.4 nm, 210.8 nm and 280.16 nm were analyzed to determine which wavelength was best suited to detect the additives. All the additives of interest could be seen in the 210.8 nm region which also gave the strongest signals.

Through trial and error, an acceptable method with the first column was created. Different aspects were analyzed during the method development, such as flow rates, eluent composition, temperatures and sample concentrations etcetera. Unfortunately, the peaks displayed slight tailing.

The method settings, from here on called Method 1, and chromatograms with the additives can be seen in appendix E:1.2. In appendix E:1.1, an early, not fully developed version of the method can be seen. Note that the concentrations of the additives are not the same in the chromatograms.

To verify the identity of the peaks, the different additives were analyzed one by one which can be seen in appendix F:1.1. Erucamide, BHT and 1,3-DTBB were also tested with the same method, to get an overview of the retention times (see appendix F:1.2).
When a satisfying method was created the C18 column was changed to a C8 column, #2, and Method 1 was used as a starting point. After a few changes of elution composition and flow rates, a method for the C8 column was created (see appendix E:1.3). With this column, good symmetry and acceptable separation of 1,3-DTBB, BHT and Erucamide were obtained. The identity of the peaks is determined in the chromatogram in appendix G:1. The method for the C8 column will hereafter be called Method 2. The used additives in Method 2 and their concentrations are:

- Irganox 1010 in IPA, 0.05 mg/g
- Irgafos 168 in IPA, 0.055 mg/g
- DEHP in IPA, 0.24 mg/g
- 1,3-DTBB in IPA, 0.09 mg/g
- BHT in IPA, 0.05 mg/g
- Erucamide in IPA, unknown concentration

### 4.2.2 Analysis of catheter extracts

The catheters in appendix A:1 were extracted in IPA as described in chapter 3.3.1. Two samples of each were done. After 24 h in a furnace at 70 °C, the samples were filtrated and then analyzed with the developed methods described in 4.2.1 and appendix E:1.2 and E:1.3.

The PEBAX catheter was totally dissolved in the extraction medium after 24 h in 70 °C. To dilute the sample in order to facilitate analysis, 5 ml IPA was added and the vial was shaken for 30 seconds. The resulting mixture was then filtrated and analyzed.

The same samples were analyzed in both methods which resulted in a delay before they were analyzed with Method 2 compared to Method 1. This may lead to differences in compositions and levels of the extracted additives although the extracts are transferred to other vessels without the catheter pieces.

Five catheters with a radiation dose of 56 kGy were extracted in MQ-water at the same conditions to see if any additive could be seen with HPLC.
4.2.3 Quantitative analysis

By constructing a calibration curve, the additives in the samples could be quantified. Only Irganox 1010 and DEHP were quantified. 1,3-DTBB can not be quantified because of suspected impurities in the standard solution. Due to that Irganox 168 are changed relatively fast to its oxidation product, it was not possible to quantify the levels. A comparison of Irgafos 168 levels over time can be seen in appendix D:1.

Five vials with five known concentrations of DEHP and Irganox 1010 were prepared and analyzed with the same methods as that used for the catheter samples. The peak area for each additive was plotted against the concentration, which gave a calibration curve. From the calibration curve, the concentrations for Irganox 1010 and DEHP in the extracted catheter samples could be calculated, appendix F:4 for Method 1 and appendix G:4 for Method 2. A mean value of the peak area for each sample type was used in the calculations.

Note that the quantitative analysis is not validated so the generated values from the quantitative analysis is not fully trustworthy. The quantitative analysis is only made to get a numerical result on what could be seen in the chromatograms.

4.2.4 Purity test of the mobile phases, MQ-water and ACN

During the method development, some unexpected peaks could be seen in all the chromatograms. To solve the problem and to find out where they came from some additional runs and experiments were done.

The first step was to be sure of no impurity in the column. ACN was washed through the column in three hours at a constant flow rate at 0.3 ml/min. After that, a sample with pure MQ-water was injected and then a sample with DEHP dissolved in IPA.

Additionally, a sample of pure IPA and ACN were injected after the sample with DEHP. This resulted in a DEHP peak in the chromatograms for these samples. This is due to the fact that DEHP are very hard to remove and it sticks very tightly to all surfaces. A three hours long wash of pure ACN at 0.3 ml/min was done to see if the column/system was contaminated, but no difference was seen after the wash. In order to get the system clean and to prevent peaks from previous sample, needle wash was tested.
During the needle wash test, three samples of pure ACN were analyzed after a DEHP sample to see if the DEHP peak would appear in the following chromatograms for ACN.

The chromatograms for the samples can be seen in appendix C:1. Timetable for the runs can be seen in appendix C:5.1.

The second step was to be sure of the purity of the solvents. This was done because of unexpected peaks at 8.5 min and at 15.7 min in all chromatograms independent of sample content. The method settings were the same as before except for the timetable, where the one in appendix C:5.2 were used. If there are peaks in the beginning of the first interval, this is cause of impurity in the column from the beginning. If the baseline drops when switching from eluent A to eluent B, this is because of impurity in eluent A. The baseline is expected to elevate. Polar impurities in the eluent B can be detecting if the baseline makes a sharp step up before flatten off. If peaks appear during the gradient when switching back to eluent A, this is because of non-polar impurities that have been accumulated in the column derived from eluent B. [29] An overview of the water purity test can be seen in figure 26.

![Figure 26. Chromatogram from water purity test.][29]

To see if the problem could be solved, a water change was done. All the MQ-water from the eluent and samples were replaced with purified water, PW-water. But the peaks continued to show up at the same retention times.

Since the peaks still appear after water change, a method with a stepwise increase of eluent B was run. This was done to see if a contamination in the system could be washed out at any other concentration of the eluent than the, so far, checked concentrations, 50 % and 100 %. The stepwise increase of eluent B can be seen in appendix C:5.3.
The same method settings were used in all the purity tests:

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume:</td>
<td>3 µl</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>0.3 ml/min</td>
</tr>
<tr>
<td>Column temperature:</td>
<td>40.0 °C</td>
</tr>
<tr>
<td>Number of injections:</td>
<td>1</td>
</tr>
</tbody>
</table>

No further experiments were done to clear up where the peaks derive from. These peaks are hereafter labeled as impurity peaks.
5 Results

The results both from FTIR and HPLC analyses of both the catheters and the extracts of the catheters are shown below. A review about the method development and the final methods are also described.

5.1 FTIR analysis

Below are the results from the FTIR analysis of various pure additives as well as catheter samples.

5.1.1 Development of FTIR spectrum of additives and constituents from standards

DEHP was easily dissolved in IPA and gave a nice FTIR spectrum with clear peaks. For the other compounds/additives the pure powder were analyzed directly on the UATR crystal. This generated weak but nice FTIR spectra. With FTIR, no difference can be seen between PVP K30 and PVP K90.

The spectra can be used as fingerprints of the additives/compounds and give a hint if specific compound is present in a sample. See appendix B:7.

5.1.2 FTIR analysis on solid catheters

No concrete deviations were seen with FTIR before and after the sterilization process for the uncoated catheters (see appendix B:2). However, a color deviation was detected in the PVC catheter on visual inspection. An attempt at identification of the different absorption maxima was also done (see appendix B:4 and A:2).

As for the uncoated catheters, no concrete deviations were seen with FTIR before and after the sterilization process for the coated PVC catheters (see appendix B:3). A color deviation was detected even here in the PVC catheter on visual inspection (see 3.3.2.3). A comparison of the FTIR spectra before and after the coating was done (see appendix B:8:1).

According to the article “PVC Stabilization during Sterilization with Electron Beam”, the reason for PVC darkening after e-beam sterilization is a catalytic process, a formation of a conjugated double bond and dehydrochlorination during the electron beam irradiation (see figure 27).
Physical properties of the catheter, such as color, can be protected by usage of antioxidants and stabilizers. [16, 30]

\[
\begin{align*}
\text{Cl} & \quad \text{hv} \\
\rightarrow & \quad \text{HCl}
\end{align*}
\]

**Figure 27.** Reaction during e-beam irradiation [30]

### 5.1.3 Extraction in MQ-water and FTIR analysis on uncoated and coated catheters

With FTIR, no substance could be seen extracted from the catheter into the MQ-water. No deviations at all were seen between the different extraction liquids for the uncoated and coated catheters independent of the different sterilization doses (see appendix B:5). No differences could be seen with a visual inspection of the extracts for the uncoated catheters but for the coated PVC catheter, the extract was turbid (see appendix A:3).

### 5.1.4 Extraction in IPA and FTIR analysis on uncoated and coated catheters

A visual inspection of the IPA samples does not show any differences from catheters extracted in MQ-water (see appendix A:4).

With use of IPA as extraction medium, substances from the catheters could be seen extracted out into the solvent in the FTIR spectra (see appendix B:6.1-5). The same applies for the coated PVC (see appendix B:6.6 and B:8.2-3). Unlike the extracts from uncoated catheters, the spectra for the coated catheters show a peak at 1650 cm\(^{-1}\). No differences could be seen before and after sterilization neither for coated or uncoated catheters.

### 5.2 HPLC analysis

From the HPLC analysis, the following results were received.

#### 5.2.1 Method development for HPLC

Two methods with different columns were developed and analyzed. Method 1 included a C18 column while Method 2 included a C8 column. Both methods used the same eluents but in different distributions. The method development was done as follows.
5.2.1.1 Method 1

Through trial-and-error, a method that separates the desirable additives was created, appendix E:1.2. DEHP and especially Irgafos 168 were tailing in the beginning but by increasing the flow rate during the retention times for this additives the tailing could be reduced to an acceptable asymmetry.

Due to that all the additives were eluted after 70 % ACN in the mobile phase, the concentration at the method start was set to 70 % of Eluent B. The fast increase, over five minutes, gave a better retention time and less tailing of the additives, but to the cost of the great separation of the additives. An even faster increase gave bad separation. However, the separation of the additives in the final method is acceptable and clearly separated from each other.

After all the additives have eluted, the eluent composition are returned to the start settings to stabilize the system for the next sample. The delay after the last additive has eluted are to stabilize the system but also to elute possible impurities and unknown additives. The impurity in the end of the analysis did not get any different elution time with a flow rate at 0.8 ml/min than with 0.3 ml/min. The flow rate was therefore decreased to 0.3 ml/min to minimize the eluent consumption.

The method temperature was set to 60 °C. This temperature gave the best retention times and helps minimize the tailing of the peaks. This is the maximum temperature of the column.

Erucamide was studied and gave a nice peak just before the DEHP elutes. BHT and 1,3-DTBB was also tried to add to the method, but because the additives has an elution time very close to each other no good separation could be done. BHT and 1,3-DTBB were also tailing and although method changes, the additive were still tailing so they were simply removed which led to that Method 1 only had the ability to separate and identify three additives correctly. All the standard solutions from the additives can be seen in appendix F:1.

5.2.1.2 Method 2

After the column change from C18 to C8, all additives could easily be separated from each other so BHT and 1,3-DTBB could also be included. This method was generally a better method for this purpose. All the peaks had good symmetry and are well separated from each other.
The change from the starting concentration of the eluent was due to that the first additives eluted were to close the solvent in retention time. The flow rates were increased momentaneously to avoid tailing and to decrease elution time.

5.2.2 Analysis of solvents from extracted catheters

The results from the HPLC analysis of the extracts from the catheters are summarized in the following table:

Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Appendix</th>
<th>PVC</th>
<th>BHT</th>
<th>1,3-DTBB</th>
<th>Eurecamide</th>
<th>DEHP</th>
<th>Irg. 1010</th>
<th>Irg. 168 ox-prod</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVC, uncoated</td>
<td>Method 1: F:2.1 Method 2: G:2.1</td>
<td>None</td>
<td>Not parsable</td>
<td>Not parsable</td>
<td>Not parsable</td>
<td>The dominating peak</td>
<td>None</td>
<td>Increase with increasing sterilization dose</td>
</tr>
<tr>
<td>PVC, coated</td>
<td>Method 1: F:2.2 Method 2: G:2.2</td>
<td>Can be seen</td>
<td>Not parsable</td>
<td>Not parsable</td>
<td>Not parsable</td>
<td>The dominating peak</td>
<td>None</td>
<td>Increase with increasing sterilization dose</td>
</tr>
<tr>
<td>POBE I, uncoated</td>
<td>Method 1: F:2.3 Method 2: G:2.3</td>
<td>None</td>
<td>Can be seen</td>
<td>Can be seen</td>
<td>None</td>
<td>None</td>
<td>Decrease with increasing sterilization dose</td>
<td>Decrease with increasing sterilization dose</td>
</tr>
<tr>
<td>POBE II, uncoated</td>
<td>Method 1: F:2.4 Method 2: G:2.4</td>
<td>None</td>
<td>Can be seen</td>
<td>Can be seen</td>
<td>Can be seen</td>
<td>None</td>
<td>Decrease with increasing sterilization dose</td>
<td>Decrease with increasing sterilization dose</td>
</tr>
<tr>
<td>PEBAX, uncoated</td>
<td>Method 1: F:2.5 Method 2: G:2.5</td>
<td>None</td>
<td>Can be seen</td>
<td>Can be seen</td>
<td>None</td>
<td>None</td>
<td>Decrease with increasing sterilization dose</td>
<td>Can be seen</td>
</tr>
<tr>
<td>Mediprene, uncoated</td>
<td>Method 1: F:2.6 Method 2: G:2.6</td>
<td>None</td>
<td>Can be seen</td>
<td>Can be seen</td>
<td>None</td>
<td>None</td>
<td>Decrease with increasing sterilization dose</td>
<td>Decrease with increasing sterilization dose</td>
</tr>
</tbody>
</table>
The different methods show the same results in differences between sterilization doses and composition of additives except from the analyzed POBE II samples. In Method 2, the POBE II sample shows a double peak at the retention time for Irganox 1010. Reasons for this may be that either the additives in the extracts are decomposed into other fragments or that the additives and fragments of additives react and form new compounds. Another reason might be that this unknown peak had the same retention time as Irganox 1010 and therefore only one peak was shown.

The samples extracted in MQ-water did not show any extracted additive with HPLC which can be compared with the FTIR analysis of the same extract. See appendix F:5 for HPLC and B:5 for FTIR. The same samples were analyzed with Method 2 after two weeks and showed some small amounts of the oxidation product from Irgafos 168 (see appendix G:5). If this is because of the duration in time or because of the change of method is unknown.

5.2.3 Quantitative analysis
As can be seen in appendix F:4.3 for Method 1 and appendix G:4.3 for Method 2, the calculated concentration of DEHP from the extracts of both coated and uncoated PVC, is outside the calibration range. For the samples containing Irganox 1010, the concentration is decreased with increasing sterilization dose. Irganox 1010 in the extracts of PVC catheters are outside the calibration range and are not reliable.

Comparison of the two methods shows that the concentrations correspond very well except for the DEHP concentration in the PVC catheters.

5.2.4 Purity test of the mobile phases, MQ-water and ACN
In the chromatogram for pure MQ-water, a peak at approximately 9.4 minutes was detected in the chromatograms at 254.4 nm, 210.8 nm and in 280.16 nm. In the chromatogram for DEHP, dissolved in IPA, a peak at approximately 8.6 minutes was detected in at 254.4 nm, 210.8 nm and in 280.16 nm. In the chromatogram at 254.4 nm, a peak at 9.4 was detected, same retention time as the great peak in the chromatogram for MQ-water (see appendix C:1).
Before the use of needle wash, samples analyzed after a DEHP sample became contaminated due to that DEHP is very hard to remove and will stick very strongly to all surfaces (see appendix C:2). This problem was not due to accumulation of DEHP in the column but because of contamination of the needle. When using the needle wash, all the following samples were clean from contamination due to the DEHP sample (see appendix C:1 and appendix C:3).

The water purity test showed a peak at 80 min due to non polar organic impurities in the MQ-water (see appendix C:4). A water change was done but the peak remained.

With the use of PW-water, the peak was still there. The stepwise increase of the eluent B, shown in appendix C:5.3 and chromatogram in appendix C:6, showed that something was eluted at 50-55 minutes thus at 70-75 % eluent B.
6 Discussion

Discussions of the obtained results from FTIR analysis, HPLC analysis and HPLC method development are described below.

6.1 FTIR analysis

FTIR works very well as a fingerprint for a sample or a specific additive. It is an easy and fast analysis and with the use of an UATR the samples are not destroyed.

6.1.1 FTIR analysis on solid catheters

Analysis on the solid catheters gave the best absorbencies, comparing to the extracted samples. Extracted samples with low concentrations of the analyte generated weak absorbencies, both for water and IPA as solvent. Comparison between the different catheters, different radiation doses and coating are discussed below.

6.1.1.1 Differences before and after radiation

With FTIR, no major differences could be seen before and after the radiation, either for the coated or uncoated catheters (see appendix B:2 and B:3). However, significant differences could be seen between the different, uncoated, catheters (see appendix B:1). The three catheters of POBE-material, POBE I, POBE II and Mediprene, are very similar to each other while the PEBAX catheter has a little more dissimilarity and the PVC catheter is quite different from the others in the absorbencies aspect. A significant difference in the absorbencies could also be seen between the coated and uncoated PVC catheter (see appendix B:8.1). The spectra for the coated catheters had a greater similarity between different catheters than was present between a coated and uncoated catheter of the same type. This is due to that the coating for the different catheters is the same and the FTIR-technique only analyzes the surface and thus does not penetrate the coating.

6.1.1.2 Differences between the catheters

IR-spectra for uncoated POBE I catheters have great similarities with spectra for uncoated POBE II catheters but also some similarities with the uncoated Mediprene catheters. This is due to the POBE-material which all these three catheters consists of (see appendix B:1).
6.1.1.3 Analysis of the different absorbencies in the spectra

See appendix B:4. An identification of peaks in reference spectra from SciFinder can be found in appendix A:2 and personally developed reference spectra can be seen in appendix B:7.

For uncoated PVC catheter, comparison with reference spectra shows that it is possible that, in addition to PVC, some peaks corresponding to DEHP can be seen in the spectra. For coated PVC catheters, mainly PVP can be seen but peaks, possibly corresponding to DEHP, can be seen even here. While comparison with DEHP spectra (appendix B:7.1) the peaks from the PVC spectra best corresponds to spectra for DEHP (see appendix B:8.5-6).

In spectra for uncoated POBE II catheter, comparison with reference spectra from SciFinder shows that it is most likely only POBE that can be seen. The spectra for the uncoated POBE II are very similar to the spectra for uncoated POBE I.

A comparison of spectra from uncoated Mediprene with reference spectra from SciFinder shows that SBS and SEBS can be seen in the spectra. However, two unidentified peaks can be seen at 1111 cm\(^{-1}\) and 1737 cm\(^{-1}\). These are probably not from SBS or SEBS. These absorbencies have been checked against spectra for Erucamide, which also exists in the catheter material, but they do not match. If Erucamide could be seen in the spectra it should give weak peaks due to the dilution factor.

When comparing the PEBAX spectra with reference spectra from SciFinder it shows that probably only PEBA can be seen in the spectra for uncoated catheter.

6.1.2 Extraction in MQ-water and FTIR analysis on uncoated and coated catheters

Nothing at all can be seen in the different FTIR spectra from the catheters extracted in MQ-water. This applies to all the samples independent of sterilization doses, coated as uncoated. See appendix B:5:1-6.
6.1.3 Extraction in IPA and FTIR analysis on uncoated and coated catheters

When extracting in IPA, some substances from the PVC and PEBAX catheter are extracted to the solvent. When comparing FTIR spectra for solid catheter with IPA-extracts of PVC and PEBAX, some of the peaks match each other. Spectra from extracts of Mediprene, POBE I and POBE II, show that something has extracted out but in a very small amount, so because of this and of interference and noise, nothing could be concluded about that. See appendix B:6 and B:8.2-4. The spectra before and after sterilization were approximately equal.

For the PVC spectra, three clear peaks can be seen (see appendix B:8.3). These three peaks may correspond to DEHP as can be seen in appendix B:8.6.

In spectra for PEBAX, a small but wide peak can be seen at 1103 cm\(^{-1}\) and a small, sharp peak can be seen at 952 cm\(^{-1}\) (see appendix B:8.4). These two peaks probably correspond to PEBA as can be seen in appendix B:4.5.

When comparing spectra from extracting medium, IPA, from coated and uncoated PVC catheter, a peak at 1650 cm\(^{-1}\) can be seen in spectra from coated PVC. This peak probably belongs to the coating of PVP and represents a C=O stretch (see appendix B:8.2 and B:4.6). A visual inspection of the coated PVC catheter samples shows turbidity as for the coated extracted in MQ-water. It also appears that the sample probably have formed some kind of colloid system.

6.2 HPLC analysis

Comparing to FTIR, the HPLC analysis gives more detailed information to the cost of the simplicity. It is easier to identify the specific compounds in a sample. While FTIR only gives a fingerprint of a sample, the HPLC can qualify, quantify and identify the additives.

6.2.1 Method development

By comparing ChemStations calculations of the peak symmetry, see appendix E:1 for the different methods it is a clear difference between the methods. For a perfectly symmetric peak, the symmetry has the value of one. The more it differs from the value one, the more the peaks are tailing. For Method 1, the symmetry values are as low as 0.41 while the lowest value for Method 2 is 0.85. In peak symmetry aspect, Method 2 is preferable.
Considering the analysis time for the methods, Method 2 is the preferred method also here. Method 1 has an analysis time of 13 minutes excluding sample injection and needle wash while Method 2 has an analysis time of 8 minutes.

### 6.2.2 Analysis of solvents from extracted catheters

From catheters of POBE material, the same additives have been extracted. Except for the concentration levels of extracted additives, the chromatograms look the same. Less Irganox 1010 has been extracted from Mediprene than from POBE I and POBE II.

Comparison between coated and uncoated PVC catheter, shows that the expected PVP peak coalesces with the solvent peak (see appendix F:3 for Method 1 and G:3 for Method 2).

Because of the total dissolvent of the PEBAX catheter in IPA, no correct conclusion about the irradiation impact and extracted amount of the additives can be made. But it can be seen that probably Irganox 1010 and Irgafos 168 is extracted out from the catheter and also something unknown just after the retention time for the solvents retention time (see appendix F:2.5 for Method 1 and G:2.5 for Method 2).

Unlike the POBE catheters, the amount of the oxidation product from Irgafos 168 is increasing with increased sterilization dose for the PVC catheters. One possible reason for that is that the peak may be coalescing with other formed compounds. A visible inspection of the peak shows that it does not look the same as the other catheter. It is irregular, which may indicate that there is other compound very close in retention time and this compound may increase with increasing sterilization dose. With increasing irradiation, the PVC gets darker which may be due to the reasoning in chapter 3.3.2.3 and this may be one other clue to the problem.

### 6.2.3 Quantitative analysis

In general, the concentration of extracted DEHP and Irganox 1010 seems to decrease with increasing sterilization dose of the catheters which also can be seen directly in each HPLC chromatogram as been discussed above. From PVC catheters, large amount of DEHP are extracted and no concrete difference between different sterilization doses can be seen.
The DEHP concentrations from the extracted PVC catheters are half the concentration in Method 2 comparing to Method 1. A possible reason for this may be that DEHP is decomposing during time since the samples were left for a week before it was analyzed with Method 2.

The concentration of Irganox 1010 in POBE II is half the concentration in Method 2 comparing to Method 1. This may be the answer to the unanswered question about the double peak. Most likely, an other unknown additive or an isomeric compound to Irganox 1010 are eluted at the same time as the Irganox 1010 in Method 1 but in Method 2 the retention time differs a little, which may result in a double peak. The identity of this peak is unknown and is not further studied.

6.2.4 Purity test of the mobile phases, MQ-water and ACN

To prevent contamination in the next sample in a series, a needle wash between each run can be preferable. Otherwise, the needle could be contaminated from compounds from previous runs. This is especially for runs on samples including DEHP, due to the sticky qualities of the DEHP.

The peak that contentiously shows up at 8 min, with current method, derives most likely from the water. Although the water was changed from MQ-water to PW-water, the peak still remained. The MQ-water and the PW-water is purified in different ways but is taken from the same tap water so that may be the reason for no difference after the water change.
7 Conclusions

FTIR works very well as a fingerprint for a sample. It is an easy and relatively fast analysis method. It is easy to detect greater differences between different samples and the different peaks can easily be detected if the sample is known. However, if the sample is completely unknown it is not so easy to detect what is the composition of the sample.

From the solid samples, analyzed with FTIR, no differences were seen before and after the sterilization. When extracting, no difference could be seen with FTIR in the extracts in MQ-water, after 24 h in 70 °C. Extraction in IPA with the same time interval and at the same temperature, gives small responses in spectra for PVC and PEBAX. While extracting PVC in IPA, DEHP extracts to the solvent and can then be detected with FTIR. No differences can be seen with FTIR before and after sterilization, either for solid samples or extracted samples. IPA as extraction medium for coated PVC catheter gives turbid solutions and the FTIR spectra shows that PVP is present in the extract.

Just as in the FTIR analysis, extracted additives can be seen in the HPLC analysis and in even more detail. With the used extraction conditions, mostly Irganox 1010 and Irgafos 168 can be seen in the POBE-catheters with IPA as extraction medium. DEHP gives very dominant peaks in chromatograms for the PVC catheters. With the used extraction conditions, the PEBAX catheter is totally dissolved and can not be correctly analyzed.

Comparison of Method 1 and Method 2 shows that Method 2 with the C8 column gives nice peaks and a better peak symmetry. Maybe with other eluents the C18 column can give better peak symmetry. With some further method development and control of repeatability and reliability, Method 2 with the C8 column may be a good analytical method for detection and quantification of extracted additives.
8 Future work

For future work, it would be interesting to try different columns and eluents to optimize the separation and to minimize the retention time. Different extraction media and extraction methods are also an aspect to look at for improvement of the methods.

The extracted compounds near the solvent peak in the HPLC chromatogram are not identified or examined. This requires changes in the method to get good separation from the solvent peak and maybe even a change in solvent or a change of the mobile phase.

The extraction method described in chapter 3.3.1 does not include some kind of stirring and some of the catheters are floating in water so the contact surface is not optimal. Thus, there is room for development of the extraction method.

The repeatability of the samples injections should be controlled. Only a few repetitions are made of each sample in this thesis due to lack of time.

There are also some unanswered questions left to answer as to why the concentration of Irgafos 168 is increasing with increasing sterilization doses for PVC catheters and why there is a double peak for Irganox 1010 in chromatograms from extracts of IPA of POBE II with Method 2.
Many thanks to Sara Richardson and Fredrik Didriksson, who gave me this opportunity to do my diploma work at Astra Tech AB.

I also want to give a special thank to my supervisor, Sara Richardson, at Astra Tech AB for all the support and help during this examination work.

Great thanks to my examiner, Ann-Sofie Cans in the division of Physical Chemistry at Chalmers University of Technology.

At last I will thank all the colleagues at R&D Urology at Astra Tech AB, for all their help and friendship.
10 References


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## Appendices

### Appendix A

1. The different catheters used in this report

<table>
<thead>
<tr>
<th>Material</th>
<th>Uncoated:</th>
<th>Coated:</th>
<th>POBE I:</th>
<th>POBE II:</th>
<th>PEBAX:</th>
<th>Mediprene:</th>
</tr>
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<td>CH 12, 40 cm</td>
<td>56 kGy</td>
<td>CH 12, 40 cm</td>
<td>100 kGy</td>
<td>CH 12, 40 cm</td>
<td>200 kGy</td>
</tr>
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<td>CH 14, 40 cm</td>
<td>100 kGy</td>
<td>CH 14, 40 cm</td>
<td>200 kGy</td>
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<td>CH 12, 40 cm</td>
<td>100 kGy</td>
<td>CH 12, 40 cm</td>
<td>200 kGy</td>
</tr>
<tr>
<td>PEBAX:</td>
<td>CH 12, 40 cm</td>
<td>56 kGy</td>
<td>CH 12, 40 cm</td>
<td>100 kGy</td>
<td>CH 12, 40 cm</td>
<td>200 kGy</td>
</tr>
<tr>
<td>Mediprene:</td>
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<td>CH 12, 30 cm</td>
<td>100 kGy</td>
<td>CH 12, 30 cm</td>
<td>200 kGy</td>
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2. Analysis of absorbance’s from FTIR spectra find at SciFinder

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<thead>
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<th>Spectrum ID</th>
<th>Absorbance wavenumbers</th>
<th>Functional groups</th>
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<td>PVC</td>
<td>BR019781</td>
<td>600-650 cm⁻¹ C-Cl</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>700 cm⁻¹ Long chain band</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1250 cm⁻¹ CH₂-Cl bend (wagging)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>1300 cm⁻¹ CH₂ bend</td>
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<td></td>
<td>1400 cm⁻¹ CH₂ bend</td>
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<tr>
<td></td>
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<td>2800-3000 cm⁻¹ C-H stretch</td>
<td></td>
</tr>
<tr>
<td>DEHP</td>
<td>BR166954</td>
<td>700 Ortho subst. oop</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1100+1250 cm⁻¹ C-O stretch</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1400-1600 cm⁻¹ Aromatic C=C stretch</td>
<td></td>
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<td></td>
<td>1700 cm⁻¹ C=O stretch</td>
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<td>BR024976</td>
<td>700 + 750 cm⁻¹ Mono subst. oop</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1600 cm⁻¹ C=C stretch vinyl</td>
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</tr>
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<td></td>
<td>2800-3000 cm⁻¹ C-H stretch</td>
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</tr>
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</tr>
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</tr>
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<td>1450-1600 cm⁻¹ Aromatic C=C stretch</td>
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</tr>
<tr>
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<td></td>
<td>1700-2000 cm⁻¹ Mono subst.</td>
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<td></td>
<td>2800-3000 cm⁻¹ sp³ C-H stretch</td>
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<td></td>
<td></td>
<td>3000-3150 cm⁻¹ sp² C-H stretch</td>
<td></td>
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<td>BR044608</td>
<td>1350 cm⁻¹ CH₃ bend</td>
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<td></td>
<td>1450 cm⁻¹ CH₂ bend</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>2800-3000 cm⁻¹ sp³ C-H stretch</td>
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</tr>
<tr>
<td>PVP</td>
<td>BR020356</td>
<td>1300 cm⁻¹ C-N stretch</td>
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<td></td>
<td>1400 cm⁻¹ CH₂ bend</td>
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<td></td>
<td></td>
<td>1600 cm⁻¹ C=O stretch</td>
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</tr>
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<td></td>
<td>2900 cm⁻¹ sp³ C-H stretch</td>
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<td>3500 cm⁻¹</td>
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<td>Erucamide</td>
<td>BR050469</td>
<td>1150 cm⁻¹ C-N stretch</td>
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<td>1350 cm⁻¹ CH₃ bend</td>
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<td>1400 cm⁻¹ CH₂ bend</td>
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<td>1600 cm⁻¹ C=O stretch</td>
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<td></td>
<td>2800-3000 cm⁻¹ C-H stretch</td>
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<td></td>
<td></td>
<td>3100 -3400 cm⁻¹ NH₂ stretch</td>
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3. Pictures – Extracted samples in MQ-water after 24 h

3:1 Catheters before extraction
From left to right: PVC, POBE I, POBE II, PEBAX, and Mediprene.

3:2 Uncoated PVC
From left to right: unsterilized, 56 kGy, 200 kGy.

3:3 Uncoated POBE I
From left to right: unsterilized, 56 kGy, 200 kGy.

3:4 Uncoated POBE II
From left to right: unsterilized, 56 kGy, 200 kGy.

3:5 Uncoated PEBAX
From left to right: unsterilized, 56 kGy, 200 kGy.

3:6 Uncoated Mediprene
From left to right: unsterilized, 56 kGy, 200 kGy.
3:7 Coated PVC

From left to right: unsterilized, 56 kGy, 100 kGy, 200 kGy.

4. Pictures – Extracted samples in IPA after 24 h

4:1 Uncoated PVC

From left to right: unsterilized, 56 kGy, 200 kGy.

4:2 Uncoated POBE I

From left to right: unsterilized, 56 kGy, 200 kGy.

4:3 Uncoated POBE II

From left to right: unsterilized, 56 kGy, 200 kGy.

4:4 Uncoated PEBAX

From left to right: unsterilized, 56 kGy, 200 kGy.
4.5 Uncoated Mediprene

From left to right: unsterilized, 56 kGy, 200 kGy.

4.6 Coated PVC

From left to right: unsterilized, 56 kGy, 200 kGy.
Appendix B - FTIR

1. FTIR-spectra for unsterilized, uncoated catheters

![FTIR spectra for various uncoated catheters](image)

- PVC catheter, uncoated, unsterilized
- POBE II catheter, uncoated, unsterilized
- Mediprene catheter, uncoated, unsterilized
- PEBAX catheter, uncoated, unsterilized

2. FTIR-spectra directly on uncoated catheters at different sterilization doses

2:1 Uncoated PVC catheter

![FTIR spectra for different sterilization doses](image)

- PVC catheter, uncoated, unsterilized
- PVC catheter, uncoated, 56 kGy
- PVC catheter, uncoated, 100 kGy
- PVC catheter, uncoated, 200 kGy
2:2 Uncoated POBE II catheter

2:3 Uncoated POBE I catheter
2:4 Uncoated Mediprene catheter

2:5 Uncoated PEBAX catheter
3. FTIR-spectra directly on coated catheters at different sterilization doses

3:1 Coated PVC catheter

4. Identification of absorption peaks in FTIR-spectrum

4:1 Unsterilized, uncoated PVC catheter
4:2 Unsterilized, uncoated POBE II catheter

4:3 Unsterilized, uncoated POBE I catheter
4:4 Unsterilized, uncoated Mediprene catheter

4:5 Unsterilized, uncoated PEBAX catheter
4:6 Unsterilized, coated PVC catheter

5. FTIR spectra of extracting medium, MQ-water, from extracted catheters

5:1 Uncoated PVC catheter
5:2 Uncoated POBE II catheter

5:3 Uncoated POBE I catheter
5:4 Uncoated Mediprene catheter

- Mediprene, uncoated, unsterilized, extracted in Milli-Q water in 70 °C for 24 h
- Mediprene, uncoated, 56 kGy, extracted in Milli-Q water in 70 °C for 24 h
- Mediprene, uncoated, 100 kGy, extracted in Milli-Q water in 70 °C for 24 h
- Mediprene, uncoated, 200 kGy, extracted in Milli-Q water in 70 °C for 24 h

5:5 Uncoated PEBAX catheter

- PEBAX, uncoated, unsterilized, extracted in Milli-Q water in 70 °C for 24 h
- PEBAX, uncoated, 56 kGy, extracted in Milli-Q water in 70 °C for 24 h
- PEBAX, uncoated, 100 kGy, extracted in Milli-Q water in 70 °C for 24 h
- PEBAX, uncoated, 200 kGy, extracted in Milli-Q water in 70 °C for 24 h
5:6 Coated PVC catheter

![](image1)

---

PVC, coated, unsterilized, extracted in Milli-Q water in 70 °C for 24 h
PVC, coated, 56 kGy, extracted in Milli-Q water in 70 °C for 24 h
PVC, coated, 100 kGy, extracted in Milli-Q water in 70 °C for 24 h
PVC, coated, 200 kGy, extracted in Milli-Q water in 70 °C for 24 h

---

6. FTIR spectra of extracting medium, IPA, from extracted catheters

6:1. Uncoated PVC catheter

![](image2)

---

PVC, uncoated, unsterilized, extracted in IPA in 70 °C for 24 h
PVC, uncoated, 56 kGy, extracted in IPA in 70 °C for 24 h
PVC, uncoated, 100 kGy, extracted in IPA in 70 °C for 24 h
PVC, uncoated, 200 kGy, extracted in IPA in 70 °C for 24 h
6:2 Uncoated POBE II catheter

- POBE II, uncoated, unsterilized, extracted in IPA in 70 °C for 24 h
- POBE II, uncoated, 56 kGy, extracted in IPA in 70 °C for 24 h
- POBE II, uncoated, 100 kGy, extracted in IPA in 70 °C for 24 h
- POBE II, uncoated, 200 kGy, extracted in IPA in 70 °C for 24 h

6:3 Uncoated POBE I catheter

- POBE I, uncoated, unsterilized, extracted in IPA in 70 °C for 24 h
- POBE I, uncoated, 56 kGy, extracted in IPA in 70 °C for 24 h
- POBE I, uncoated, 100 kGy, extracted in IPA in 70 °C for 24 h
- POBE I, uncoated, 200 kGy, extracted in IPA in 70 °C for 24 h
6:4 Uncoated Mediprene catheter

- Mediprene, uncoated, unsterilized, extracted in IPA in 70 °C for 24 h
- Mediprene, uncoated, 56 kGy, extracted in IPA in 70 °C for 24 h
- Mediprene, uncoated, 100 kGy, extracted in IPA in 70 °C for 24 h
- Mediprene, uncoated, 200 kGy, extracted in IPA in 70 °C for 24 h

6:5 Uncoated PEBAX catheter

- PEBAX, uncoated, unsterilized, extracted in IPA in 70 °C for 24 h
- PEBAX, uncoated, 56 kGy, extracted in IPA in 70 °C for 24 h
- PEBAX, uncoated, 100 kGy, extracted in IPA in 70 °C for 24 h
- PEBAX, uncoated, 200 kGy, extracted in IPA in 70 °C for 24 h
6:6 Coated PVC catheter

7. FTIR-spectra of standard solutions of additives

7:1 DEHP
7:2 Irgafos 168

7:3 Irganox 1010
8. Comparisons

8:1 Coated and uncoated PVC catheter

8:2 Comparative FTIR-spectra of extracting medium, IPA, from uncoated and coated PVC catheter and also FTIR-spectra of solid PVC catheter
8:3 FTIR spectra of extracting medium, IPA, from uncoated PVC and solid PVC catheter

8:4 FTIR spectra of extracting medium, IPA, from uncoated PEBAX and solid PEBAX catheter
8.5 FTIR spectra from solid PVC catheter and DEHP

8.6 Split FTIR spectra of extracting medium, IPA from PVC catheter and spectra for DEHP
Appendix C – Purity test of HPLC

1. Impurity peak appearing in a number of samples

1:1 HPLC-UV chromatogram from MQ-water

1:2 HPLC-UV chromatogram from DEHP in IPA
2. Appearing DEHP peak in samples without DEHP

2:1 HPLC-UV chromatogram without needle wash from pure IPA after a run with DEHP sample

2:2 HPLC-UV chromatogram without needle wash from pure ACN after a run with DEHP sample
3. A series of samples were analyzed with the use of needle wash

3:1 HPLC-UV chromatogram with needle wash from sample 1 with pure ACN after a run with DEHP sample

3:2 HPLC-UV chromatogram with needle wash from sample 2 with pure ACN after a run with DEHP sample
3:3 HPLC-UV chromatogram with needle wash from sample 3 with pure ACN after a run with DEHP sample

4. Water purity test

4:1 Water purity test at 254.4 nm
4:2 Water purity test at 210.8 nm

5. Eluent distributions

5:1 Eluent distribution for the draft method
5:2 Eluent distribution for purity test method

Timetable: Eluent distribution

5:3 Eluent distribution for stepwise gradient method

Timetable: Stepwise increase of eluent B
6. Stepwise gradients

Absorbance at 254.4 nm.

6:1 Stepwise gradient from 55 % eluent B to 100 % eluent B

6:2 Stepwise gradient from 70 % eluent B to 80 % eluent B
Appendix D - Analysis difficulties

1. Multiple liquid chromatograms over time

Absorbance at 210.8 nm.

1:1. Irgafos 168 and its oxidation product

1:2. Irgafos 168

1:3. Oxidation product of Irgafos 168
2. Chromatogram of PVP K90, PVP K30 and IPA

Absorbance at 210.8 nm.
Appendix E – HPLC

1. Chromatogram of additives, values and method settings at 210.8 nm

1:1 Chromatogram of additives with unfinished method for C18 column

<table>
<thead>
<tr>
<th>#</th>
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Solvent
- Ox-prod of Irgafos 168
- Irganox 1010
- DEHP

Irganox 1010 in IPA, 0.06 mg/g
Irgafos 168 in IPA, 0.15 mg/g
DEHP in IPA, 0.12 mg/g

Method settings

- Injection volume: 3 μl
- Column temperature: 40 °C
1:2 Chromatogram of additives with finished method for C18 column: Method 1

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Irganox 1010 in IPA, 0.05 mg/g
Irgafos 168 in IPA, 0.11 mg/g
DEHP in IPA, 0.24 mg/g
1:3 Chromatogram of additives with finished method for C8 column: Method 2

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**Method settings**

Eluent B
- Concentration (% ethanol): 0 to 100
- Flow rate (mL/min): 0 to 3

Flow rate
- Concentration (% ethanol): 0 to 100
- Flow rate (mL/min): 0 to 3

Injection volume: 3 µL
- Column temperature: 50 °C
Appendix F – HPLC: Method 1

1. Method 1: Chromatograms of pure additives

Absorbance at 210.8 nm.

1:1 Chromatograms of different additives

- Irganox 1010
- Irgafos 168
- DEHP

1:2 Chromatogram of all additives

- Irganox 1010
- Irgafos 168
- DEHP
- Ox-prod Irgafos 168
- Erucamide
- BHT
- 1,3-DTBB
- Impurities
2. Method 1: Chromatograms of catheter extracts, 24 h at 70 °C

Absorbance at 210.8 nm.

Symbols:
1. Solvent
2. DEHP
3. Irganox 1010
4. Irgafos 168
5. Irradiation product of Irgafos 168
6. Oxidation product of Irgafos 168
7. Unknown
8. PVP
9. Impurity

2:1 Uncoated PVC catheter
2:2 Coated PVC catheter

Reference chromatogram
- PVC, coated, unsterilized, extracted in IPA
- PVC, coated, 56 kGy, extracted in IPA
- PVC, coated, 100 kGy, extracted in IPA
- PVC, coated, 200 kGy, extracted in IPA

2:3 Uncoated POBE I catheter

Reference chromatogram
- POBE I, uncoated, unsterilized, extracted in IPA
- POBE I, uncoated, 56 kGy, extracted in IPA
- POBE I, uncoated, 100 kGy, extracted in IPA
- POBE I, uncoated, 200 kGy, extracted in IPA
2:4 Uncoated POBE II catheter

Reference chromatogram
- POBE II, uncoated, unsterilized, extracted in IPA
- POBE II, uncoated, 56 kGy, extracted in IPA
- POBE II, uncoated, 100 kGy, extracted in IPA
- POBE II, uncoated, 200 kGy, extracted in IPA

2:5 Uncoated PEBAX catheter

Reference chromatogram
- PEBAX, uncoated, unsterilized, extracted in IPA
- PEBAX, uncoated, 56 kGy, extracted in IPA
- PEBAX, uncoated, 100 kGy, extracted in IPA
- PEBAX, uncoated, 200 kGy, extracted in IPA
3. Method 1: Comparison between coated and uncoated PVC catheters, extraction in IPA

Absorbance at 210.8 nm.
4. Method 1: Qualitative analysis

4:1 Calibration curve for DEHP

4:2 Calibration curve for Irganox 1010
### Concentration calculations

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- outside the calibration range
- inside the calibration range
5. Method 1: Extraction of uncoated catheters in MQ-water

Absorbance at 210.8 nm.

- PVC, uncoated, 100 kGy, extracted in Milli-Q water
- PEBAX, uncoated, 100 kGy, extracted in Milli-Q water
- Mediprene, uncoated, 100 kGy, extracted in Milli-Q water
- POBE I, uncoated, 100 kGy, extracted in Milli-Q water
- POBE II, uncoated, 100 kGy, extracted in Milli-Q water
Appendix G – HPLC: Method 2

1. Method 2: Chromatogram of pure additives

Absorbance at 210.8 nm.
2. Method 2: Chromatograms of catheter extracts, 24 h at 70 °C

Absorbance at 210.8 nm.

Symbols:  
1. Solvent  
2. DEHP  
3. Irganox 1010  
4. Irgafos 168  
5. Irradiation product of Irgafos 168  
6. Oxidation product of Irgafos 168  
7. Unknown  
8. PVP  
9. Impurity

2:1 Uncoated PVC catheter
2:2 Coated PVC catheter

2:3 Uncoated POBE I catheter
2:4 Uncoated POBE II catheter

2:5 Uncoated PEBAX catheter
2:6 Uncoated Mediprene catheter

3. Method 2: Comparison between coated and uncoated PVC catheters, extraction in IPA

Absorbance at 210.8 nm.
4. Method 2: Qualitative analysis

4:1 Calibration curve for DEHP

\[ y = 3.4888x \]
\[ R^2 = 0.9999 \]

Concentration [µg/g] vs. Area [mAU*s]

4:2 Calibration curve for Irganox 1010

\[ y = 5.4661x \]
\[ R^2 = 0.9948 \]

Concentration [µg/g] vs. Area [mAU*s]
## 4.3 Concentration calculations

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</tbody>
</table>

- outside the calibration range
- inside the calibration range
5. Method 2: Extraction of uncoated catheters in MQ-water

Absorbance at 210.8 nm.

- PVC, uncoated, 100 kGy, extracted in Milli-Q water
- PEBAX, uncoated, 100 kGy, extracted in Milli-Q water
- Mediprene, uncoated, 100 kGy, extracted in Milli-Q water
- POBE I, uncoated, 100 kGy, extracted in Milli-Q water
- POBE II, uncoated, 100 kGy, extracted in Milli-Q water