Lipid deposition on contact lenses when using contemporary care solutions

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Abstract

Purpose: To determine the effect of lens care system combinations on levels of total lipid, cholesterol and cholesteryl esters extracted from three different contact lenses when used with four contemporary care systems.

Methods: Experienced contact lens wearers were recruited to participate in this study. Combinations of three contact lens materials (etafilcon A, galyfilcon A and senofilcon A) and four contact lens care solutions (Biotrue, ClearCare, OPTI-FREE PureMoist and RevitaLens Ocutec) were investigated. A total of 791 contact lenses were analyzed. Subjects were randomized to one lens type and then used all four lens care solutions in random sequence for 10-14 days before the contact lenses were collected and analyzed for the amount of cholesterol, cholesteryl esters and total lipids, which were extracted using liquid chromatography and mass spectrometry.

Results: The mean range of cholesterol recovered across the different care solutions was 0.34-2.77 μg/lens, 3.48-4.29 μg/lens and 4.75-6.20 μg/lens for etafilcon A, senofilcon A and galyfilcon A lenses, respectively. Use of OPTI-FREE PureMoist with etafilcon A lenses led to a significantly greater amount of cholesterol being recovered when compared to the use of the other solutions with etafilcon A lenses (p<0.05). The mean range of cholesteryl esters recovered across different care solutions was 1.31-2.02 μg/lens, 6.43-7.19 μg/lens and 7.96-10.13 μg/lens for etafilcon A, senofilcon A and galyfilcon A lenses, respectively. There were no differences in the amount of cholesteryl esters and total lipids extracted for a given lens type when used with any of the four care solutions (p>0.05).
Conclusions: This study did not demonstrate conclusively that any of the solution/contact lens combinations were superior to any of the other combinations, when the amounts of lipid deposition were compared among the tested lenses. The amount of lipid uptake by contact lenses is influenced by the chemical composition of the contact lens material.

Keywords: contact lens; deposition; lipids; lens care solution
Introduction

The tear film consists of water, ions, proteins and lipids. Tear film lipids are produced by the meibomian glands, which are specialized holocrine glands that line both the upper and lower eyelids.¹ Meibomian gland fluid consists of both polar and non-polar molecules and includes steroids, glycerides, saturated and unsaturated fatty acids, fatty acid polar esters and polar lipids.² Non-polar lipids comprise the outer tear film layer and overly the polar lipids, which act as the intermediate phase between the aqueous layer and non-polar lipid layer and aid in tear film stability.³ Disorders of the meibomian glands or lipid layer of the tear film can lead to numerous ocular disorders, with dry eye being the major complication reported.⁴

The impact of tear film lipids on contact lens wear has been studied for several decades.⁵⁻¹³ Lipid deposition may impact contact lens comfort, clarity of vision and potentially initiate an inflammatory state, secondary to lipid degradation.¹²,¹⁴ There has been some speculation that oxidation of lipids may be contributory to end of day contact lens discomfort¹⁴⁻¹⁶ and lipids appear to be degraded to greater amounts in overnight versus daily wear of contact lenses.¹⁴ Longer replacement periods resulted in increased lipid deposition.¹⁷,¹⁸ Initial studies on soft contact lens lipid deposits described the appearance of "jelly bumps", "lens calculi" or "mulberry spots", which were found to be principally made of cholesteryl esters.⁵,¹⁹,²⁰ The role of lipid deposits on the health of the ocular surface has also been investigated, as more solution induced corneal staining was observed in patients with greater amounts of lipid deposits.²¹

Recently, there has been a greater emphasis on the relevance of lipid deposition on soft contact lenses due to the commercialisation of silicone hydrogel lenses. This is because of the inclusion of silicon moieties that improve oxygen transmission with silicone hydrogel lenses when
compared with conventional hydrogel materials.\textsuperscript{22-24} As a result, silicone hydrogel lenses are more hydrophobic,\textsuperscript{22, 24-26} and more prone to lipid deposition. Deposition of total lipid, cholesterol, cholesterol esters and triglycerides/phospholipids on contact lenses has been investigated.\textsuperscript{27} A silicone hydrogel material (balafilcon A) deposited higher levels of lipid when compared to a hydrogel material (etafilcon A) after 10 hours of daily disposable or 7 days of extended wear, demonstrating that lipid deposition occurs relatively rapidly.\textsuperscript{27} A full understanding of the total lipid deposited on worn lenses has been complicated by the large variation in reported levels of deposition between authors, using different quantification techniques. Enzymatic sulpho-phospho-vanillin and enzymatic oxidation were used to demonstrate higher levels of cholesterol deposition (20-30 μg/lens) on worn lenses when compared with other lipids.\textsuperscript{28} In that same study, the levels of total lipid deposition was 33-42 μg/lens.\textsuperscript{28} Nash and colleagues, using a fluorescent enzymatic assay, also showed significant differences in lipid deposition on worn silicone hydrogel materials, with maximum lipid deposition being approximately 4 μg/lens.\textsuperscript{12} Both of these studies are in contrast to earlier studies, which utilized high performance liquid chromatography (HPLC) to quantify up to 600 μg/lens of certain lipids, although the authors have recently quantified 40 μg/lens of lipid deposition on lenses and suggested this amount may be more accurate.\textsuperscript{7, 8, 11}\par Recent studies have investigated the impact of care solutions on removal of lipids deposited on contact lenses. One study, that investigated the deposition and removal of cholesterol and dipalmitoylphosphatidylcholine (DPPC) using commercial solutions or borate buffered saline, coupled with simulated rubbing, reported that only a small amount of cholesterol and no DPPC was removed by these solutions.\textsuperscript{10} Cheung \textsl{et al.}\textsuperscript{29} used atomic force microscopy to visualize the deposits on the surface of lenses soaked in artificial tear solution and indicated that after cleaning
contact lenses using a hydrogen peroxide or polyhexamethylene biguanide solution, only trace visible amounts of the tear fluid components remained on the lens surface.\textsuperscript{29}

The design of modern contact lens care solutions is complicated by the multiplicity of roles that need to be performed by a single solution. A single solution is expected to clean, condition and disinfect worn contact lenses, and also be compatible with the ocular surface. Furthermore, the majority of lenses are marketed as being compatible and appropriate for all soft contact lens types. However, there remains only a small amount of data from human studies demonstrating the effect of lens/solution combinations on the deposition of lipids.\textsuperscript{30-32} Considering the potential importance of contact lens lipid deposits on vision and comfort, information on the degree to which modern materials deposit when used with contemporary care solutions is needed.

The aim of this \textit{ex vivo} study was to determine the effect of lens care system combinations on levels of total lipid, cholesterol and cholesteryl esters extracted from three different contact lenses when used with four contemporary care systems.

\textbf{Materials and Methods}

\textbf{Participants}

This trial was conducted in compliance with the Declaration of Helsinki and participants signed the consent form prior to enrollment in the study. Experienced spherical soft contact lens wearers \((n=236)\) between the ages of 18 and 69 years (inclusive) were enrolled across three investigational sites (The University of Houston, Houston, Texas, USA, University of Manchester, Manchester, United Kingdom and the University of Waterloo, Waterloo, Ontario, Canada). There were no restrictions as to gender or race/ethnicity. This study reports the data collected from only
two sites (University of Waterloo and the University of Houston), due to logistical complications related to the shipment of lenses to the USA from the UK site.

**Contact Lenses and Care Solutions**

The lenses used in this study were etafilcon A (Acuvue 2), galyfilcon A (Acuvue Advance Plus) and senofilcon A (Acuvue Oasys) (all Johnson & Johnson Vision Care, Jacksonville, Florida). As needed, contact lens wear in the washout periods was accomplished using etafilcon A with Lacreon technology (1-Day Acuvue Moist) (Johnson & Johnson Vision Care, Jacksonville, Florida). The properties of the lenses used in this study are presented in Table 1. A total of four contact lens care solutions were investigated in this study. Detailed properties of the four solutions investigated are presented in Table 2.

Each participant received a product information form which had instructions on the proper use of the dispensed solutions as per each manufacturer’s instructions. For the preserved solutions, participants were instructed to rub their lenses for 2-4 sec and rinse for 5 sec using RevitaLens; rub their lenses for 20 sec and rinse for 10 sec using PureMoist; rub their lenses for 20 sec and rinse for 5 sec using Biotrue, before placing them into lens cases for overnight soaking. When ClearCare was used, no rub was required but participants were instructed to rinse their lenses for 5 sec when the lens was in the lens holder and then fill the lens case before overnight soaking.

**Study Design and Wear Schedule**

Each subject was randomly allocated to wear one of the three lens types after a washout period wearing either spectacles or 1-Day ACUVUE Moist (1DAVM). Once assigned, each participant utilized each of the four investigational solutions over a period of 10-14 days in a randomized sequential fashion, with a minimum wear time of 8 hours per day, and 8 total days of
contact lens wear in that period. Between solutions, there was a washout period of at least 4 days with spectacle or 1DAVM wear.

Subjects were masked to the lens type being used and the solutions were de-identified as much as possible, while the clinical investigator(s) were masked as to the type of care solution that participants used. At the end of each lens-solution wear period (contact lenses were collected at scheduled visits), the clinical investigator, wearing latex-free gloves, removed the lens from the participant's left eye and placed it directly in a dry 7 ml polypropylene vial. Each contact lens was stored individually and the vials were sealed prior to storage at -80˚C until analysis took place. The lens-containing vials were placed in cryoboxes and subsequently in Styrofoam filled completely with dry ice before shipping them to Johnson & Johnson Vision Care Inc. (Analytical Research & Development, Jacksonville, Florida) for the analysis of cholesterol, cholesteryl esters and total lipid amounts. Lenses collected from the participants' right eye were used for protein analysis and the results from this portion of the study have been published previously.33

Lipid Analysis in Contact Lens Extracts

Reagents

Dichloromethane (EMD Millipore Corp, VWR, Atlanta, Georgia, USA), scintillation vials (VWR, Atlanta, Georgia, USA), cholesteryl-2,2,3,4,4,6-d₆ octadecanoate (CDN Isotopes, Pointe-Claire, Quebec, Canada), cholesterol-2,2,3,4,4,6-d₆ (CDN Isotopes, Pointe-Claire, Quebec, Canada), heptane (EMD Millipore Corp, VWR, Atlanta, Georgia, USA), isopropanol (EMD Millipore Corp, VWR, Atlanta, Georgia, USA) and cholesteryl linoleate (Sigma-Aldrich, St. Louis, Missouri, USA) were used in this study.
Preparation of Lipid Working Standards

A stock solution of cholesteryl linoleate and cholesterol was prepared in diluent (70% heptane and 30% isopropanol) at 1000 µg/mL and 500 µg/mL, respectively. A series of working standards with concentrations of 0.5, 1.0, 5.0, 10.0, 15.0, and 20.0 µg/mL for cholesteryl linoleate and 0.25, 0.50, 2.5, 5.0, 7.5 and 10.0 µg/mL for cholesterol were prepared from the stock solution. Each working standard also contained cholesteryl-2,2,3,4,4,6-d<sub>6</sub> octadecanoate and cholesterol-2,2,3,4,4,6-d<sub>6</sub> at the 2.5 µg/mL level.

Lipid Extraction

Cholesteryl esters and cholesterol were extracted from contact lenses using dichloromethane in 20 mL glass scintillation vials. To each vial, contact lens, 5 mL of dichloromethane and 100 µL of an internal standard solution were added. The internal standard solution consisted of cholesteryl-2,2,3,4,4,6-d<sub>6</sub> octadecanoate and cholesterol-2,2,3,4,4,6-d<sub>6</sub> (CDN Isotopes) each at the 25 µg/mL level in diluent (70% heptane and 30% isopropanol). Lenses were extracted for a minimum of 16 hours at 3°C. Extracts were then transferred to a 5 mL disposable glass culture tube and evaporated using a TurboVap® LV Concentration Evaporator Workstation (Biotage, Charlotte, NC). Dried extracts were immediately reconstituted with 1 mL of diluent and carefully transferred to Thermo autosampler vials for Liquid Chromatography-Mass Spectrometry analysis. When necessary, lens extracts were further diluted in order to measure the sample within the range of the working standard calibration curve. Unworn lenses were used as controls and specificity was demonstrated during method qualification.

Liquid Chromatography-Mass Spectrometry (LC-MS)

A Thermo Accela high performance liquid chromatography (HPLC) system coupled on-line to a Quantum Ultra EMR triple quadrupole mass spectrometer (Thermo Scientific, San Jose,
CA) was used for the quantification of lipids in contact lens extracts. The mass spectrometer was equipped with an atmospheric pressure chemical ionization (APCI) source. The LC separation was performed on an Agilent Zorbax NH₂ column (4.6 x 150 mm; 5 µm particle size). The isocratic elution method consisted of 70% heptane and 30% isopropanol with a flow rate of 1000 µL/min. The column temperature was set at 30°C and the injection volume was 25 µL. Thermo X-Calibur software was used for data acquisition and analysis. The mass spectrometry operation parameters were as follows: positive ion mode (APCI+); scan mode, SIM at m/z 369.2 for [cholesterol – H₂O + H]+ and m/z 375.3 for [cholesterol(d₆) – H₂O + H]+; mass width, m/z 2.0; scan time, 0.15s; spray voltage, 4000V; skimmer offset, 10V; capillary temperature, 200°C; vaporizer temperature, 450°C; tube lens offset; 117V; sheath gas pressure, 20 units; auxiliary gas pressure, 15 units.

**Statistical Analysis**

This paper presents only a sub-set of data that involved the evaluation of various clinical signs, symptoms, protein and lipid deposition and bacterial contamination of lens cases. In this ancillary study, a total of 791 contact lenses (253 etafilcon A, 280 senofilcon A and 258 galyfilcon A lenses) were analyzed. Previous studies on lipid deposition on contact lenses used substantially smaller sample sizes (maximum of 30 contact lenses). Therefore, the sample size for this study was considered sufficient to explore differences in lens care solutions.

Levels of lipid extracted from worn contact lenses were analyzed to test for the differences between the care solutions. The outcome variables were cholesterol, cholesterol esters and total lipid (the summation of cholesterol and cholesteryl esters) in µg/lens units. Data were log transformed before analysis. Each parameter was analyzed using a linear mixed model to test for the difference between the contact lens care solutions within each lens type. An unstructured (UN)
covariance matrix was used to model the correlation between measurements within the same subject/eye across visits. All statistical tests were 2-sided and conducted at the 5% level of significance. Adjustment for multiple pairwise comparisons between solutions across lens types was conducted using a Bonferroni correction. Data analysis was conducted using Statistica 12 (Statsoft Inc, Tulsa, Oklahoma) and SPSS 22 (International Business Machines Corporation, Armonk, New York) software programs.
Results

Cholesterol and cholesteryl esters were not detected in the unworn control lens extracts. A total of 791 contact lenses (368 lenses from Houston and 423 lenses from Waterloo) including 253 etafilcon A, 280 senofilcon A and 258 galyfilcon A were analysed for lipid deposition. Table 3 provides a summary of the amount of cholesterol, cholesteryl esters and total lipids (μg/lens) extracted from contact lenses.

Cholesterol

Figure 1 (a-c) and Table 3 reports the amounts of cholesterol extracted from etafilcon A, senofilcon A and galyfilcon A contact lenses when used in combination with the various care solutions. Higher amounts of cholesterol were extracted from senofilcon A and galyfilcon A lenses than etafilcon A lenses ($p<0.05$). Additionally, results indicated that levels of cholesterol extracted from etafilcon A lenses were higher with OPTI-FREE PureMoist than with the other lens care solutions (all, $p<0.05$). There were no differences in the amounts of cholesterol extracted from senofilcon A and galyfilcon A lenses when used with the various care solutions ($p>0.05$).

Cholesteryl Esters

Figure 2 (a-c) and Table 3 demonstrates that higher amounts of cholesteryl esters were extracted from senofilcon A and galyfilcon A lenses compared to etafilcon A lenses ($p<0.05$). There were no significant differences in the amounts of cholesteryl esters extracted from a given lens type when used with the different care solutions ($p>0.05$).

Total Lipid

The amount of total lipid in this study was defined as the sum of cholesterol and cholesteryl esters (two major lipid deposits on contact lenses). Figure 3 (a-c) and Table 3 show that higher
amounts of total lipids were extracted from senofilcon A and galyfilcon A lenses than etafilcon A lenses \((p<0.05)\). There were no significant differences in the amounts of total lipid extracted from a given lens type when used with the various care solutions \((p>0.05)\).

**Discussion**

In this study, the care solutions investigated generally behaved similarly in their ability to affect the recovery of cholesterol, cholesteryl esters and total lipids from worn lenses. The only significant difference seen was in the amount of cholesterol removed from etafilcon A materials when used with OPTI-FREE PureMoist. In all other cases, the amount of cholesterol, cholesteryl esters and total lipids recovered were not statistically different for a given lens type when used with the different solutions. The reduced ability of OPTI-FREE PureMoist to remove cholesterol from etafilcon A-based materials during wear may be due to an interaction of the material and the composition of OPTI-FREE PureMoist, which uniquely contains TETRONIC 1304, EOBO-41 (HydraGlyde Moisture Matrix), sorbitol, and aminomethylpropanol. However, further work would be required to elucidate the repeatability of this finding, its etiology and whether such a small difference is clinically relevant.

The mean range of cholesterol recovered across the different care solutions was 0.34-2.77 μg/lens, 3.48-4.29 μg/lens and 4.75-6.20 μg/lens for etafilcon A, senofilcon A and galyfilcon A lenses, respectively. Cholesterol is one of the most widely investigated tear lipid deposits on contact lenses. In a study by Tam et al.\(^{38}\) the *in vitro* sorption of cholesterol onto various contact lenses including senofilcon A was investigated, and the ability of both Biotrue and OPTI-FREE PureMoist in removing the lipid were explored. Treatment of senofilcon A materials for 16 h in artificial tear solution followed by the exposure of the lenses to either of the two solutions for 8 h
led to less than 1% removal of the deposited cholesterol. They reported values on senofilcon A ranging from 5.5-8.0 μg/lens depending on the laboratory condition and incubation solution used. They reported values on senofilcon A ranging from 5.5-8.0 μg/lens depending on the laboratory condition and incubation solution used. The amounts of cholesterol recovered from the aforementioned in vitro study were higher than the amounts of cholesterol recovered from the current ex vivo study, which could be due to the use of different quantification methods employed in these two studies (radiolabelling in Tam et al. study versus LC-MS technique in the current study) or the fact that one study was an in vitro study and the other examined ex vivo lenses.

In an ex vivo study, Zhao et al. investigated the use of different solutions (OPTI-FREE Express, AQuify, ClearCare and OPTI-FREE RepleniSH) on the deposition and removal of cholesterol from galyfilcon A and senofilcon A lenses. Between the two lenses, the greatest amount of cholesterol was recovered from galyfilcon A (6.4 ± 0.2 μg/lens) materials cleaned with OPTI-FREE Express, while only 2.7 ± 0.8 μg/lens was recovered from senofilcon A materials using the same solution. The amounts of lipid recovered in that study are in the range of the amounts of cholesterol recovered in the current study using different care solutions. Nash et al. utilized a fluorometric enzymatic assay to determine the amount of cholesterol deposited on lenses ex vivo. The lenses analyzed were gathered from eight different clinical trials in the USA and Australia. Galyfilcon A and senofilcon A lenses were used with ClearCare or AOSEPT (Alcon, Fort Worth, Texas), which are both hydrogen peroxide-based systems. They reported 2.80 ± 0.8 μg/lens and 3.75 ± 1.1 μg/lens of cholesterol sorption by senofilcon A and galyfilcon A lenses respectively. In the current study when ClearCare was used, 4.29 ± 4.85 μg/lens and 4.75 ± 2.54 μg/lens of cholesterol was recovered from senofilcon A and galyfilcon A lenses respectively, which is higher than the amounts found in the Nash et al. study. Use of different quantification techniques could once again be the reason for the discrepancy in results between these two studies.
The mean range of cholesteryl esters recovered across different care solutions was 1.31-2.02 μg/lens, 6.43-7.19 μg/lens and 7.96-10.13 μg/lens for etafilcon A, senofilcon A and galyfilcon A lenses, respectively. Total cholesterol esters deposited on etafilcon A after 7 days of extended wear reported by Maissa et al.\cite{27} was 3.39 ± 2.63 μg/lens, suggesting that there might be more deposition of cholesterol esters when the lenses are worn on an extended wear basis, wherein they are not subjected to cleaning using a lens care system. Pucker et al.\cite{39} developed an enzymatic method to determine *ex vivo* and *in vitro* levels of cholesterol and cholesterol esters deposition on lotrafilcon B and galyfilcon A contact lenses.\cite{39} Using this method, they determined that galyfilcon A deposited 5.77 ± 1.87 μg/lens when worn with either OPTI-FREE Express or OPTI-FREE RepleniSH.

Hatou et al.\cite{28} studied the total amount of lipid, phospholipid and cholesterol deposited on galyfilcon A, senofilcon A and asmoofilcon A lens materials. There was no restriction on multipurpose care solution types that could be used by the patients during the two weeks of the study. Hatou et al.\cite{28} did not find a significant difference in the amount of total lipid or cholesterol deposited on the galyfilcon A or senofilcon A lenses, although the amounts of lipids reported were significantly higher than what is reported in the current study, with total lipid and cholesterol being estimated to be 32.9 ± 33.8 μg/lens and 26.2 ± 26.9 μg/lens for galyfilcon A and 42.1 ± 14.0 μg/lens and 28.6 ± 19.4 μg/lens for senofilcon A.\cite{28} The large difference between the two studies may be due to the difference between the characteristics of the study populations, differences in care solutions used and differences in the lipid extraction and recovery methods used (enzymatic and colorimetric probes for Hatou et al. versus LC-MS used in the current study).

Using a sulfo-phospho-vanillin assay, Mochizuki et al.\cite{40} also measured the amount of total lipids extracted from contact lenses. They found that total lipid deposition was greater in the
polymacon group (66.3 ± 16.3 µg/lens) than in the etafilcon A group (44.1 ± 8.2 µg/lens). The amount of total lipid in the current study ranged from 1.40 ± 1.01 to 2.11 ± 2.38 µg/lens on etafilcon A when RevitaLens OcuTec and OPTI-FREE PureMoist care solutions were used respectively, which is much lower than that reported by Mochizuki and co-workers. This difference could be due to study populations or the use of different measurement methodology in the studies (sulfo-phospho-vanillin assay for Mochizuki et al. versus LC-MS in the current study).

In conclusion, this study did not demonstrate conclusively that any of the solution/contact lens combinations were superior to any of the other combinations, when the amounts of lipid recovered were compared among the tested lenses. This limits the ability to make specific recommendations regarding optimal lens care solution-material combinations for wearers. Additional studies are required to investigate whether the nature of all lipid deposits on lenses is detrimental. It remains a possibility that certain lipid deposits onto lenses may allow for greater comfort during wear, and once identified, selective removal of lipids and other deposits by the next generation of solutions may be advisable.

Acknowledgements

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References


**Figure legends:**

**Figure 1 (a-c):** Mean ± SD amounts of cholesterol (log μg/lens) extracted from contact lenses when used with different care solutions

**Figure 2 (a-c):** Mean ± SD amounts of cholesteryl esters (log μg/lens) extracted from contact lenses when used with different care solutions

**Figure 3 (a-c):** Mean ± SD total lipid (log μg/lens) extracted from contact lenses when used with different care solutions
Table 1: Contact lenses used in the study

<table>
<thead>
<tr>
<th>Contact Lens</th>
<th>WASHOUT LENSES</th>
<th>RANDOMIZATION LENSES</th>
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<td>1-Day Acuvue Moist</td>
<td>Acuvue 2</td>
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<td>Johnson &amp; Johnson Vision Care, Inc.</td>
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<td>8.3 8.3 8.4</td>
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<td>Material</td>
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<td>etafilcon A galyfilcon A senofilcon A</td>
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<td>Solution</td>
<td>Biotrue</td>
<td>ClearCare</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
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<tr>
<td>Manufacturer</td>
<td>Bausch + Lomb</td>
<td>Alcon Vision Care</td>
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<td>Preservatives/Disinfecting Agents</td>
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<td>Hydrogen Peroxide 3%</td>
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<td>Other Components</td>
<td>Hyaluronan, sulfobetaine, poloxamine, EDTA, sodium chloride</td>
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<td>Buffer</td>
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<td>Phosphonic Acid/Sodium Phosphate</td>
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Table 3: Summary of cholesterol, cholesteryl esters and total lipids (μg/lens) extracted from contact lenses with different care solutions. 253 etafilcon A, 258 senofilcon A and 280 galyfilcon A were analyzed for lipid deposition.

<table>
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<tr>
<th>Contact lens</th>
<th>Solution</th>
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<th>Cholesteryl esters</th>
<th>Total lipid</th>
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