

# *Collection Forum*

## **ALCOHOL RECYCLING AT THE SMITHSONIAN INSTITUTION, NATIONAL MUSEUM OF NATURAL HISTORY (NMNH)**

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*Society for the Preservation  
of Natural History Collections*

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*Abstract.*—In an attempt to reduce the volume of hazardous waste generated by specimen processing and curation activities, the Smithsonian Institution, National Museum of Natural History evaluated a solvent/formalin recycler. The purpose of the evaluation was to produce a contaminant-free recycled alcohol product for reuse in specimen curation. Over 40 test samples of used alcohol (isopropanol and ethanol) from various fluid-preserved zoological specimens were tested. The distillation of each 13–17 L used alcohol sample required 5–9 hours to complete, yielding recycled alcohol at 89–95% concentration. A significant odor, probably derived from amines, not specifically identifiable through chromatography or other methods, could be detected in the recycled ethanol. Molecular analysis of a spiked alcohol sample both before and after distillation showed that DNA does not survive the distillation process. Pre- and postdistillation samples were analyzed by gas chromatography–mass spectrometry (GC–MS). The GC–MS results for ethanol routinely identified the presence of ethyl ethers, ethyl esters, and aldehydes, all in very small concentrations. These compounds were present both before and after distillation, with little change in concentration. Arene compounds, including toluene and xylenes, also were routinely identified in the isopropanol solutions both before and after distillation.

## INTRODUCTION

Chemical recycling regularly occurs in medical histological laboratories and manufacturing plants (Pinizzotto and Baker 2000; Hampton 2007). It is not used widely in natural history museums, but could be a tremendous cost saver by reducing the amount of new alcohol purchased and hazardous waste generated. In general specimen curation and processing activities, the Smithsonian Institution, National Museum of Natural History (NMNH) consumes 11,945 L of ethanol and isopropanol a year at an annual cost of \$12,661.64. The NMNH also generates 4,789 L of hazardous waste alcohol a year at an annual total cost of \$4,070.65 to process.

The NMNH evaluated a solvent/formalin recycler as part of a study to reduce the volume of new alcohol consumed and hazardous waste generated by specimen processing and curation activities. The recycler was assessed to determine its effectiveness and feasibility for general museum use by producing a cost-effective, contaminant-free, recycled alcohol for reuse in general specimen curation. The recycled alcohol needs to be chemically and molecularly pure enough to be reused without contaminating any specimens that it contacts. The goal was to take any alcohol sample, even those with high levels of contaminants, and provide an end product that was as clear and odorless as a purchased product.

## MATERIALS AND METHODS

### *Recycler*

Fluid samples from collections of the NMNH were gathered to test a commercially available 19-L capacity SLV–99U(L) model fractional distillation Formalin/Solvent

Recycler. Due to the small quantity of formalin-archived collections and resultant waste produced annually by our departments, it was decided to only test the solvent (alcohol) component. The test samples included almost all of the major phyla that comprise the fluid collections at NMNH, covering a wide spectrum of contaminants derived from the fixing/preservation of these taxa as well as the chemicals derived from the reaction of the specimens with the preservative fluid. The unit was programmed with heat settings designed for use with isopropanol and ethanol. One program temperature (85°C) was set up for the recycling of high-concentration fluids (>65% alcohol) with a second program using a higher temperature (90°C) to derive the same results for starting solutions with a concentration of <65% alcohol. These settings provided us with a clear product between 89% and 95% concentration and a waste product that was approximately 30% alcohol. Pre- and postdistillation 2-oz (59.2 ml) samples were taken and the volume and alcohol percentage were recorded. Any special characteristics of the predistillation samples or end product were also recorded, generally to remark on the odors that survived the distillation process.

One alcohol pretreatment test was performed prior to distillation. The alcohol was acidified with concentrated sulfuric acid to a pH of 5 before recycling. To allow complete reaction of any amines in the alcohol with the sulfate ion, forming insoluble salts, the pretreatment sample sat for 24 hours and then was distilled in the recycler.

A number of alcohol posttreatment tests were performed. Large quantities of activated charcoal were poured into containers of recycled ethanol, agitated, and allowed to sit for 12 hours. The product poured off was clouded by precipitate from the charcoal but the odor was noticeably lessened. The fluid then was poured through a funnel lined with coffee filters to remove the charcoal dust and recycled a second time. Additionally, two activated charcoal filtration columns were designed for posttreatment of the recycled alcohol. The columns were constructed from 4 ft (1.2 m) tall, 4 in. (10 cm) diameter PVC pipe with a finely perforated cap, lined with four paper coffee filters, packed tightly with granular activated charcoal, and set up with an unused glass catch. A 19-L carboy of recycled ethanol and isopropanol was then placed over each dedicated charcoal column, respectively, and set to a slow drip. The slow drip allowed the fluid a maximum amount of time in contact with the charcoal and prevented fine charcoal particulate in the catch.

#### *GC-MS Analysis*

Alcohol samples were run using an Agilent 6890 gas chromatograph with 5975 quadrupole mass spectrometer and an Agilent 7694E headspace analyzer (GC-MS). A number of sample pretreatments were tried, including adding sodium chloride to the alcohol solutions and extracting with chloroform or diethyl ether; however, no pretreatments showed improved volatilization of any compounds in the GC-MS chromatograms. Aliquots of the alcohols collected both pre- and postdistillation were placed in headspace vials, filling half of the vial. Samples were run on two different GC columns. An Agilent J&W DB-WAX, 30 m × 0.25 mm × 0.50 μm column maximized identification of the reactive aldehydes and amines. However, because the large volumes of ethanol and isopropanol present in the samples coeluted on the DB-WAX column, samples were also run on the Agilent J&W HP-5MS, 30 m × 0.25 mm × 0.50 μm column to provide information on the relative amounts of ethanol and isopropanol in each sample.

Prior to headspace extraction, vials were held at 50°C for 5 minutes. The sample loop was filled for 0.2 minutes at 55°C and the transfer line to the GC was held at 60°C.

Helium carrier gas was used at a constant flow rate of 0.8 ml/min. A split/splitless inlet was used in split mode at a temperature of 70°C with a 20 : 1 split. The column was held at 25°C for 5 minutes, then heated at 10°C/min to 150°C and held for 5 minutes. The transfer line to the MS was at a temperature of 200°C. The mass spectrometer used electronic ionization, with the ion source at 230°C and the quadrupole at 150°C, measuring masses from 20 to 300 m/z (mass-to-charge ratio).

The peaks in the chromatogram were deconvoluted and identified using both retention time (RT) and the mass spectrum by the AMDIS software program, the NIST MS Search program and the NIST05 library produced by the National Institute of Standards and Technology, Gaithersburg, Maryland. Quantification of any of the compounds was not attempted—too many unknown variables made this difficult—and analysis of the data only established approximate amounts of the compounds.

### *Molecular Analysis*

Because amplifiable DNA is known to leach into alcohol storage solutions (Shokralla et al. 2010) and would contaminate subsequent nondestructive sampling of preservative if not eliminated, we tested for survival of DNA posttreatment. Positive control fish DNA was extracted from a commercially obtained fresh fillet of yellowfin tuna (*Thunnus albacore*) using a phenol-chloroform protocol on the Autogenprep 965 automated DNA extractor (Autogen, Holliston Massachusetts). The concentration of the DNA extract was 39 ng/μl with a spectrophotometer 260/280 absorbance ratio of 1.78. After adding 1 mg of tuna DNA to the 2.75 gal (10.4 L) of clear 96% ethanol, a 50 ml pretreatment sample was taken. The ethanol recycler ran on the 85°C program and an additional 50 ml posttreatment sample was taken after the run was completed.

Pre- and posttreatment samples were prepared for polymerase chain reactions (PCR) to amplify DNA. To test for starting template quantity variation, 10 μl, 100 μl, 1.5 ml, 3 ml, and 9 ml ethanol was evaporated, eluted in 10 μl molecular-grade water, and 1 μl of this was used as template in the PCR reactions. Then, 1.35 ml ethanol was precipitated with 150 μl, 3 M ice-cold sodium acetate (NaOAc), and 7.5 μl 2 M magnesium chloride (MgCl<sub>2</sub>). The pellet was eluted in 10 μl molecular grade water, and 1 μl was used as template in the PCR amplification to detect DNA.

The PCR was one cycle of 30 seconds at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 52°C, 45 seconds at 72°C, and a final extension of 300 seconds at 72°C. The procedure was performed on an Applied Biosystem 2720 Thermal Cycler (an exception to this protocol was an annealing temperature of 48°C for the primer pair of dgLCO1490/ dgHCO2198).

The 10 μl reaction volume contained 1 μl template DNA, 1.0 μl 10× PCR Buffer, 0.4 μl 50 mM MgCl<sub>2</sub>, 0.5 μl 10 mM dNTPs, 0.3 μl 10 mM each of forward and reverse primers, and 0.5 U *Taq* DNA polymerase (reagents: BioLine USA Inc., Taunton, Massachusetts). Negative and positive controls were included with each reaction.

## RESULTS AND DISCUSSION

### *Recycler*

A total of 35 ethanol and 10 isopropanol samples were run through the recycler. Regardless of initial concentration of alcohol, the recycler yielded recycled alcohol at 89–95% concentration by volume. The distillation time and return volume was proportional to the starting fluid concentration (Figs. 1, 2). The waste product created by the recycler was approximately 30% alcohol, a level considered to be hazardous waste.

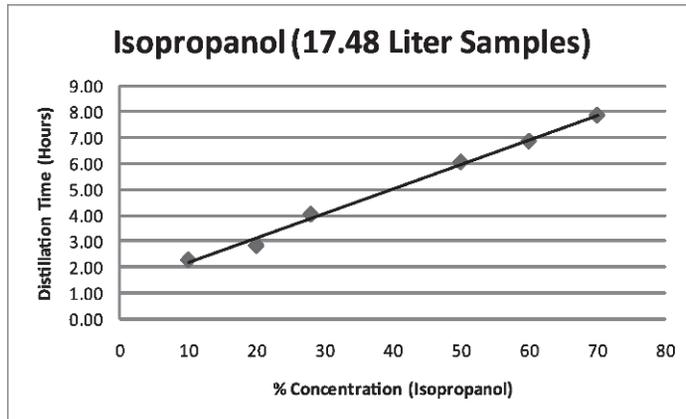


Figure 1. Graph of distillation times and concentration return from isopropanol samples.

The initial samples tested were discolored, clouded with precipitates, and extremely odiferous. The goal was to produce a final product that would be up to archival collections standards. Despite the high levels of dyes, precipitates, and other contaminants, the recycler was able to produce an end product that was as clear as commercially available chemical grade ethanol or isopropanol (Fig. 3). However, the odors in the recycled fluid were equal to the original product or, in some cases, more concentrated. The odors were very strong for the ethanol batches but were perceived to be weaker after distillation of the isopropanol samples. This was not considered significant due to the sharp odor of pure isopropanol and the likelihood that this masked any perception of the odors.

The odors were thought to be amines; a variety of methods were attempted to solve this problem. The recycler was reprogrammed with different temperatures and modes designed to leave the odor-causing compounds behind in the waste product, but none of the new temperature settings were able to achieve this result.

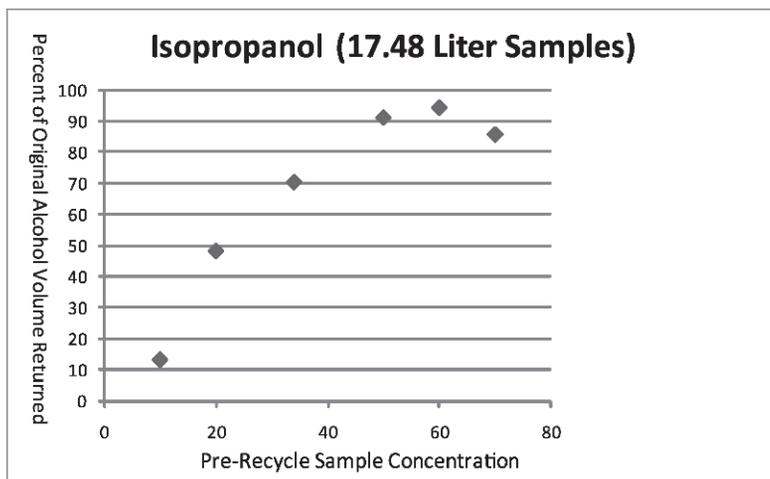


Figure 2. Graph of pre- and postrecycling concentration results for isopropanol.

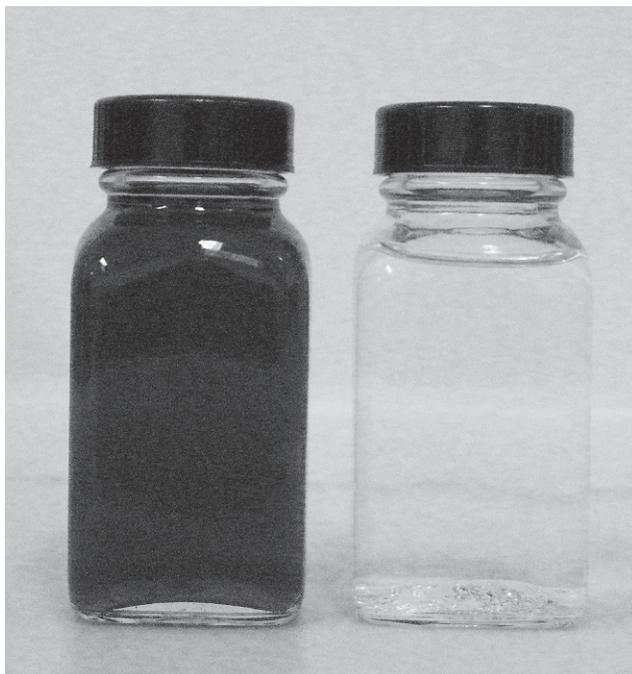


Figure 3. Comparison of pre- (left) and post- (right) distillation alcohol.

The average pH of the alcohol samples was 7. Lowering the pH to 5 with sulfuric acid could result in the odor-causing amines and other impurities precipitating out of solution. A fine precipitate did result from these tests, but the odors still remained after distillation.

One source of contamination was the recycler itself. Some contaminants were surviving the distillation process, remaining inside the unit, and contaminating each subsequent test. To provide the most accurate posttreatment samples, the unit was cleaned after each sample run by running a batch of 95% pure ethanol through a complete distillation. Even after two cleaning runs of 95% ethanol, the end product still retained the odors imparted by previous contaminated samples.

Activated charcoal commonly is used to remove odors and other contaminants. Several methods were attempted to maximize the effect and the time of contact between the fluids and the charcoal. Simply mixing activated charcoal with recycled alcohol and allowing the mixture to sit for 12 hours produced a good but not perfect result; residual odors remained.

When the activated charcoal was used in the PVC filtration column, it resulted in a clear and odorless alcohol product. Each column worked well for 50 L of fluid before any odor could be noticed. The lifespan of the columns was longer if the charcoal was tightly packed and the flow from the source carboy was kept to a slow drip. Any attempt to speed up the process created preferential flow channels in the charcoal and shortened the contact time between the fluid and the charcoal, which allowed contaminants to remain in the alcohol.

#### *GC-MS Analysis*

*Known samples.*—The samples of pure ethanol and isopropanol each contained a small amount of other alcohols. The isopropanol contains approximately 0.02–0.08% 1-

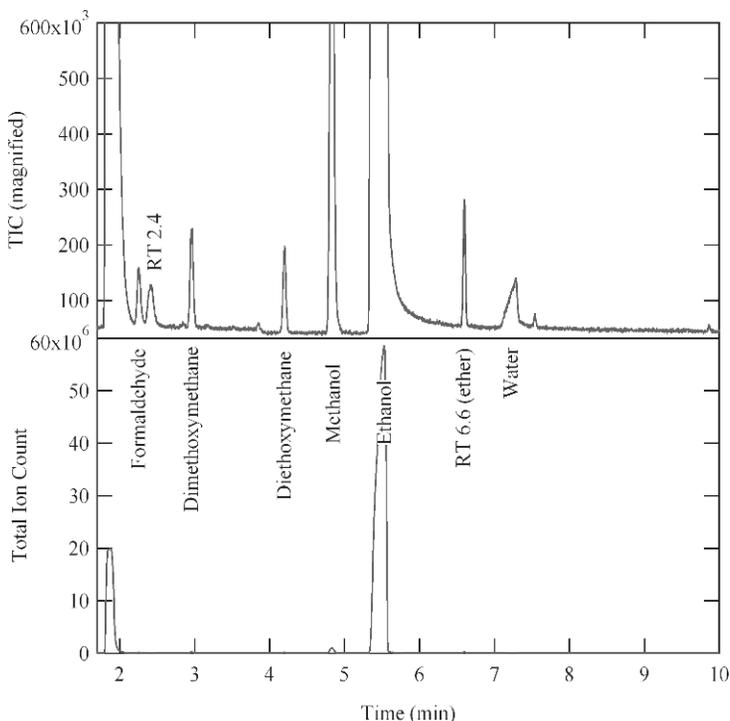


Figure 4. Chromatogram of 1% formaldehyde solution on DB-WAX column.

propanol; the ethanol contains approximately 0.1–0.4% isopropanol and approximately 20–80 ppm of methanol.

When a solution of 10% formalin (3.7% formaldehyde in ethanol and water) was run on the GC–MS, a number of compounds were identified. Formaldehyde and methanol both were present in the chromatogram, along with dimethoxymethane, diethoxymethane, two unknown ethers at retention time (RT) 6.59 and RT 10.84, and an unknown compound at RT 2.4 (Fig. 4).

However, when a more dilute sample of formalin was prepared, the formaldehyde was below the limit of detection for the GC, approximately 0.5%.

*Ethanol samples.*—Twenty-three ethanol sets (pre- and postdistillation samples) were evaluated with GC–MS.

Five of these sets were pure alcohol run through the distiller to check for contamination of the recycler that might carry across alcohol sets. Of the eighteen storage ethanol sample sets, more than half contained ethyl acetate, acetal, acetone, 1,1-diethoxyethane, methanol, ethyl formate, and diethoxymethane (Table 1). A number of compounds were found more rarely: other ethyl esters, ethyl ethers, and aldehydes (Table 1). Two ethanol samples, ALC0048 (fish source) and ALC0068 (exhibit crab source), showed trimethyl amine, a notoriously fishy smelling amine. All of the contaminants identified in the ethanol samples were present in small amounts. In one of the most contaminated sample sets, ALC0009 and ALC0010, the total of all contaminants identified was 0.39% of the alcohol peak before distillation (approximately 0.20% of the overall starting solution). The total of all contaminants detected by GC

Table 1. Compounds identified in ethanol storage samples: 3/4, 9/10, 13/14, 39/40, 43/44, 47/48, 51/52, 55/56, 59/60, 67/68, 71/72, 73/74, 75-77, 78/79, 80/81, 82/83, 84/85, 86-91. \*Identified with HP-5MS column, not used for samples 80-91; † RT = Retention Time.

Name	Compound class	Total sets	Found before and after distillation	Only after distillation	Only before distillation
Acetal (acetaldehyde)	Aldehyde	18	18	—	—
1,1-Diethoxyethane	Ether	17	15	1	1
Acetone	Ketone	16	10	6	—
Ethyl acetate	Ester	15	10	5	—
Ethyl formate	Ester	14	6	4	4
Methanol	Alcohol	13	11	2	—
Diethoxymethane	Ether	12	11	1	—
Isopropanol*	Alcohol	12	12	—	—
Unknown (RT 6.59)†	Ether	8	6	2	—
1,1-Diethoxypropane	Ether	8	4	2	2
Ethyl butanoate	Ester	5	3	1	1
Toluene	Arene	4	3	—	1
2-Butanol	Alcohol	4	—	—	4
Propanal	Aldehyde	4	2	2	—
<i>m</i> -Xylene	Arene	3	3	—	—
1-Propanol	Alcohol	2	1	—	1
2-Butanone	Ketone	2	2	—	—
Ethylbenzene	Arene	2	—	—	2
Methyl 2-methyl-2-propenoate	Ester	2	1	1	—
Ethyl 2-methylbutanoate	Ester	2	2	—	—
Hexanal	Aldehyde	2	1	—	1
Unknown (RT 10.86)†	Ether	2	2	—	—
Trimethyl amine?	Amine	2	—	2	—
<i>p</i> -Xylene	Arene	1	1	—	—
3-Methylbutanal	Aldehyde	1	—	—	1
Ethyl hexanoate	Ester	1	1	—	—
Ethyl 2-methyl-2-propanoate	Ester	1	1	—	—
Unknown (RT 2.4)†	Unknown	1	—	1	—

increased to 0.95% of the alcohol peak after distillation, with the largest individual contaminant, acetal, being 0.36% of the alcohol peak (Figs. 5, 6).

*Isopropanol samples.*—Six isopropanol storage samples were analyzed. Acetone and 1-propanol are present in all the samples, with 2-butanone, ethanol, toluene, ethylbenzene, xylenes, acetaldehyde, 2-methylpropyl acetate, and methyl 2-methyl-2-propenoate found in half or more of the samples (Table 2).

*Pure alcohol samples.*—The five pure ethanol samples were very clear. Small amounts of methanol and isopropanol were present in all samples. After the samples were run through the distiller they typically remained quite clear. However, looking specifically for isopropanol and its common aromatic contaminants, xylenes and ethyl benzene, in ALC0038 small amounts of isopropanol (shown by ion 59) and xylenes (shown by ion 106) from the previous run remained in the distiller and contaminated the next batch of ethanol run through the distiller (Fig. 7).

*Specially treated alcohol samples.*—One batch of ethanol, ALC0075, was acidified with sulfuric acid to a pH of 5, ALC0076, and subsequently distilled, ALC0077. The fish odor was slightly reduced but not eliminated, and GC-MS analysis showed that the

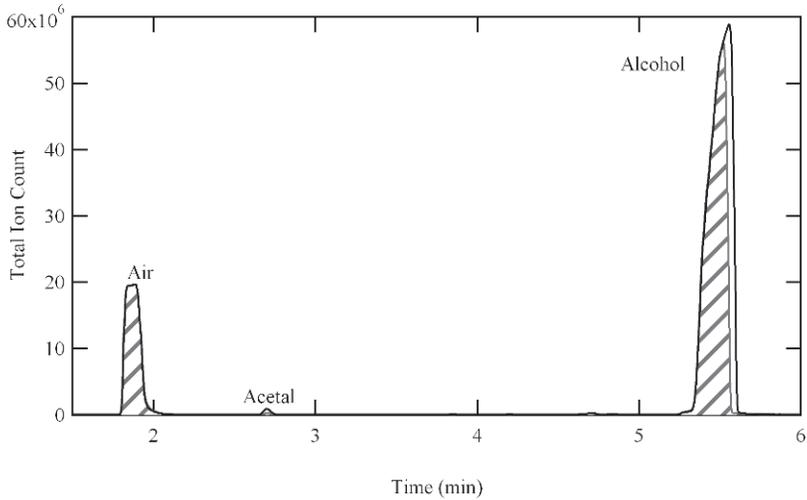


Figure 5. Chromatograms from ALC0009 (shaded) and ALC0010 (black) on DB-WAX column.

majority of compounds identified in the starting solution also remained after acidification and distillation.

Two sets of alcohol, one ethanol (ALC0086-91) and one isopropanol (ALC0092-94) were further purified after distillation by running the distilled alcohol through a packed column of activated charcoal. The charcoal initially was effective at completely reducing the fishy odor. After 50 L of isopropanol were run through the packed charcoal column the column became ineffective and the fishy odor was no longer removed from the isopropanol, ALC0094. When the samples were analyzed with GC-MS the first

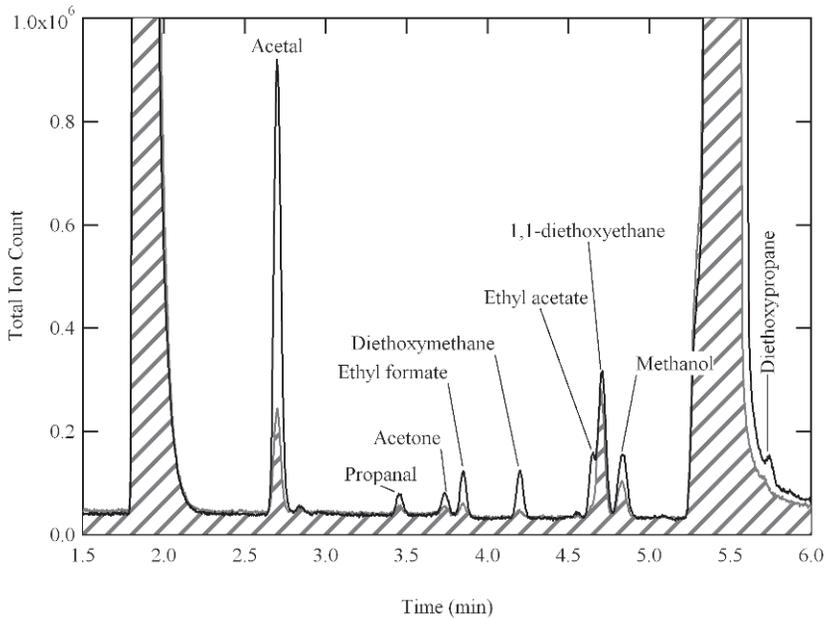


Figure 6. Chromatograms from ALC0009 (shaded) and ALC0010 (black), Figure 5, magnified. Note that many compounds actually increased in the postdistillation sample, ALC0010.

Table 2. Compounds identified in isopropanol storage samples: 17/18, 19/20, 23/24, 27/28, 69/70, 92–94. \*Identified with HP-5MS column, not used for samples 92–94.

Name	Compound class	Total sets	Found before and after distillation	Only after distillation	Only before distillation
Acetone	Ketone	6	6	—	—
1-Propanol	Alcohol	6	6	—	—
2-Butanone	Ketone	5	5	—	—
Ethanol*	Alcohol	5	5	—	—
2-Methylpropyl acetate	Ester	4	1	3	—
Acetaldehyde (acetal)	Aldehyde	3	1	2	—
Toluene	Arene	3	1	2	—
<i>m</i> -Xylene	Arene	3	2	1	—
Ethylbenzene	Arene	3	2	1	—
Methyl 2-methyl-2-propenoate	Ester	3	3	—	—
<i>o</i> -Xylene	Arene	3	1	2	—
<i>p</i> -Xylene	Arene	3	1	2	—
Ethyl acetate	Ester	2	1	1	—
Benzene*	Arene	2	2	—	—
Ethyl formate	Ester	1	1	—	—
2-Methyl-1-propanol	Alcohol	1	1	—	—
2-Methyl-3-pentanone	Ketone	1	1	—	—
Diisopropyl ether (isopropyl isopropane)	Ether	1	1	—	—
Formaldehyde	Aldehyde	1	—	—	1
4-Methyl-2-pentanone	Ketone	1	1	—	—
<i>n</i> -Pentanol	Alcohol	1	1	—	—

isopropanol run through the column (ALC0092) still showed the presence of acetone and methanol, although the isopropanol looked and smelled cleaned. GC–MS analysis of ALC0094 showed a larger amount of acetone and methanol than was present in the first sample passed through the charcoal column.

### *Molecular Analysis*

Molecular analysis of a spiked alcohol sample, both pre- and postdistillation, showed that no detectable DNA survived the distillation process and no DNA from the spiked DNA sample was detectable by amplification (Fig. 8).

### CONCLUSIONS

Alcohol recycling, on principle, is a valid method to reduce the amount of purchased alcohol and the waste product generated. The unit, however, failed to produce a recycled product that met collections archival standards for reuse in multiple disciplines without having potential cross contamination or inherent contaminants from the used fluid source. The cost effectiveness for a museum becomes irrelevant if the end product is unusable for general specimen curation. The Smithsonian rarely produces a large scale, single source recycling event. A proposed use of the recycled fluid is in soaking out formalin in recently fixed specimens as they transfer to the standard preservation fluids of 50% isopropanol and 70% ethanol. This still leaves the disposal cost for that volume of fluid after the process for each batch of specimens, because the waste produced is at a concentration level that is still considered to be hazardous waste.

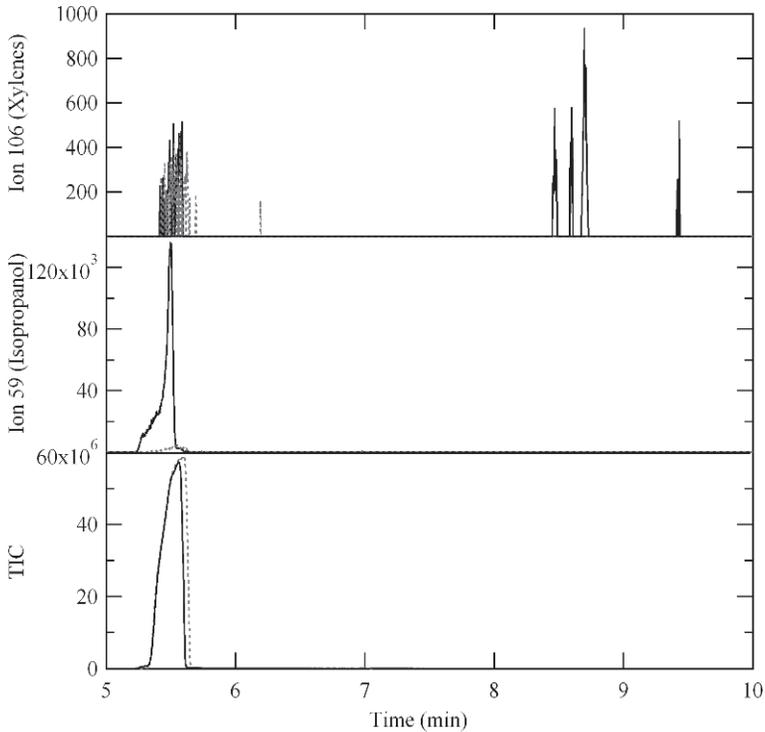


Figure 7. Chromatogram (TIC) and extracted ion chromatograms for isopropanol (59) and xylenes (106) for clear ethanol samples ALC0037 (dashed) and ALC0038 (solid). Note that both isopropanol and the xylenes only are present after distillation, ALC0038.

Alcohol recycling is effective in producing a visually clear fluid that is free of contamination from DNA. However, GC-MS shows the samples still retain a number of chemical contaminants, including cross-contamination between batches of alcohol. This might be acceptable if the sample being recycled is being reused in the original container and is not derived from multiple sources of used alcohol. If the material to be recycled is from multiple sources and multiple phyla, then the final product will be a cumulative solution of all of those chemicals. Introducing this product back into a specimen jar might be detrimental to those specimens.

The unit tested did not provide an odor-free product. An odor-free product was only achieved by constructing a custom filtration column of activated charcoal, which was costly and doubled the total distillation time. Although the odor was reduced, some chemical contaminants remained in the alcohol after charcoal filtration. After a maximum of 50 L, the charcoal in the column would need to be replaced, a significant expense in cost and time with the only result being that the final product does not have a residual smell.

As museum research moves from being libraries of morphologically variant specimens to being repositories of material for DNA, proteomic, or other types of biochemical analysis, formalin and other sample cross-contamination is of paramount importance. Formalin is not completely removed by any of the processes explored in this article. Just as cross-contamination is possible between batches of isopropanol and ethanol, cross-contamination by formalin from batch to batch also could be possible.

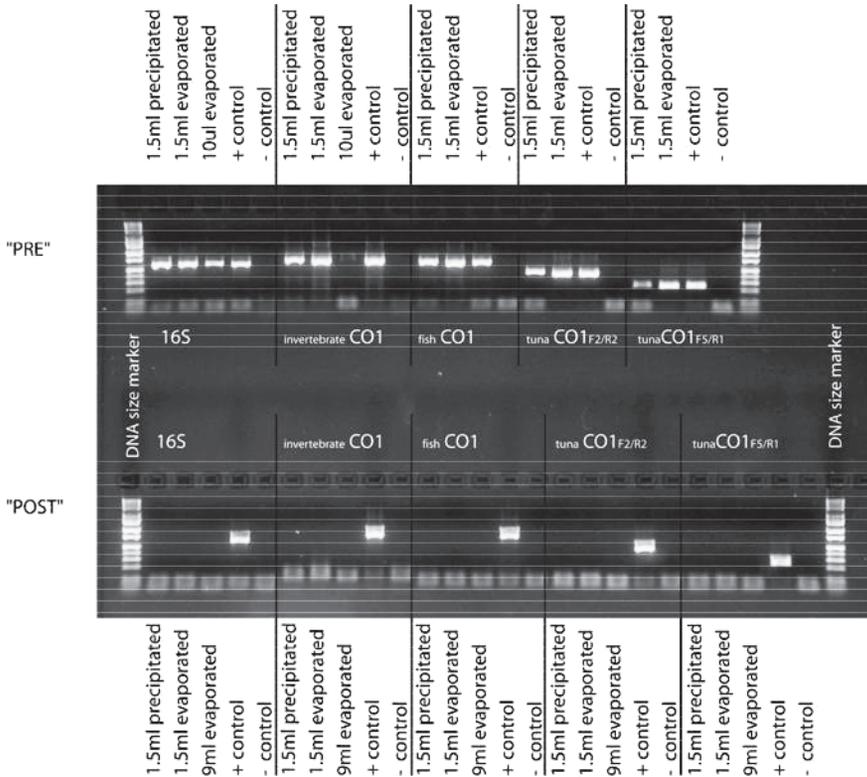


Figure 8. Comparison of PCR amplification of pre- and posttreatment samples with positive controls, negative controls, and DNA size marker. The different template quantity variations and loci with primer sets used are shown.

Storage in alcohol over many years results in reaction between the specimens and the alcohol that produces contaminants with a similar enough volatility to the alcohol that temperature distillation, even combined with charcoal filtration, was unable to produce a satisfactory recycled alcohol end product. Testing of the solvent/formalin recycler has shown that the final product cannot be considered for use in specimen preservation because it does not satisfy many of the requirements in producing a pure chemical-grade product. The solutions produced and the concentration of contaminants is highly variable. Pure ethanol and isopropanol could not be produced, even when the starting solution was fresh from an unused drum of 95% ethanol or 99% isopropanol. Because the final product is unreliable for general curation and the cost of the unit to process the fluid is so high, it is not feasible to purchase a solvent recycler for use in museum collections. The only reliable source of alcohol is still the chemical distributor.

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