

Tritium Analysis of Burn-Derived Water from Natural and Petroleum-Derived Products

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A practical means of using tritium to authenticate a natural versus a synthetic (petroleum-derived) origin of food and flavoring extracts has been demonstrated. Extracts derived from natural botanicals reflect a tritium content incorporated during the plant's growth stages, while petroleum-derived products, because of the relatively short half-life of tritium, have no measurable tritium content. Natural and petroleum-derived samples of the common flavorant benzaldehyde were combusted by Parr bomb and enriched in tritium using an alkaline electrolysis technique. Tritium activities were measured using an ultra-low-level liquid scintillation counter, with the results demonstrating a clear difference in the tritium content of natural and petroleum-derived benzaldehyde samples and a good agreement between the natural sample and the steady-state concentration of tritium reported for the northern hemisphere.

Keywords: *Tritium; tritium enrichment; alkaline electrolysis; natural product authentication; botanical extracts*

INTRODUCTION

A method of determining the age of carbon-containing materials based on radiocarbon (^{14}C) content was proposed in 1955 by Libby. The method depends on continuous production of ^{14}C in the environment and its transport into living material. Using this method, compounds derived from plants exhibit "modern" levels of ^{14}C , whereas petroleum-derived versions of the same compounds exhibit measurably lower levels of ^{14}C —often zero levels. Thus, the distinction between "natural" and "petroleum-derived" has been determined and has been used to validate claims of natural origin for foods and flavor extracts (Noakes and Hoffman, 1980; Martin et al., 1980).

Kaufman and Libby (1954) reported that tritium is continuously produced in the troposphere and, because of radioactive decay, continuously depleted. Tritium in contact with water vapor rapidly exchanges with the water protons, forming tritiated water, HTO, at the interface of the moist (lower) and the dry (upper) layers of the troposphere. Because the decay and the production rates are equal, a steady-state concentration of tritium in the environment exists and is given an average value of 4.5–5.4 TU (tritium units = 1 part in 10^{18}) or 14.6–17.5 pCi/L for the northern hemisphere (Okai et al., 1994). Precipitation and evaporation cycle tritium between the atmosphere and surface water with fractionation that favors HTO buildup in the liquid phase or surface water. Variations in the so-called steady-state tritium concentration are observed in surface waters (Brown and Grummit, 1956; Matsuoka, 1992).

The range of tritium concentration in surface waters after the mid-1950s has been reported in the range of 110–335 pCi/L (Stewart, 1967). Current values are lower because atmospheric tritium releases have ceased. Because atmospheric nuclear testing was carried out

primarily in the northern hemisphere, the tritium levels in the southern hemisphere were not artificially elevated in the 1950s and have remained fairly constant.

Botanical metabolic reliance on surface water as its ultimate proton source results in incorporation of tritium from tritiated water as well as other available protons in accordance with natural abundance of the hydrogen isotopes. During growth of the plant, tritium atoms are incorporated into selected proton positions on the metabolic product, according to the dynamics of its biosynthesis. Tritium atom exchange with labile protons is another mechanism that incorporates tritium into plants during growth, in particular, and after growth, incidentally (Stewart, 1967; Streitwieser and Heathcock, 1981). Thus, plants tend to exhibit steady-state tritium concentration in their metabolic products; predictably, plant extracts exhibit a tritium burden that reflects tritium exposure during growth.

Because tritium reaches its half-concentration in a little over 12 years, petroleum-derived products have no measurable tritium content. Thus, by measuring tritium concentration in alleged botanical extracts, a basis of distinguishing between natural and petroleum-derived products exists. Moreover, a false or valid claim of natural origin for a given product may be determined by such means.

Methods for plant extract authentication that rely on deuterium concentration, ^{14}C analysis (carbon dating), and $\delta^{13}\text{C}$ (0/00) determination exist. As each of these methods was developed, strategies for adulteration to defeat the analytical method were devised. Because of the increased number and different approaches of the analytical methods, the new adulteration strategies were increasingly complex and difficult to employ (Culp et al., 1990).

The purpose of this study was to demonstrate another practical means of authenticating the origin of proton-rich chemicals and develop guidelines for such analysis that obviates false negatives and false positives. Benzaldehyde was selected as the model compound for this work because both known petroleum-derived and natu-

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ral examples were available and also because of its importance as a flavorant.

MATERIALS AND METHODS

Apparatus. The tritium measurements for this study were performed by an ultra-low-background liquid scintillation counter (LSC), the Wallac Quantulus ultra-low-level LSC, Model 1220. The instrumental parameters employed on the LSC were based on maximizing the value of the E^2/B ratio, where E is the counting efficiency and B is the background (cpm). It is usually the case that when the counting efficiency is maximum, so is the background. Referring to eq 4, by algebraically manipulating the expression for the minimum detectable activity (MDA), it can be shown that the "best" values for background and efficiency are those that result in the largest value for E^2/B —which also results in the smallest/lowest MDA for a particular counting experiment. Data used to calculate tritium activity were derived from tritium counting windows adjusted to maximize E^2/B . Although not fundamentally necessary, as a rule, once these windows were set, they were not changed during the course of the experiment. The practice was justified because we counted samples that were of a fairly constant volume and composition.

The instrument gain was set according to a similar strategy; the gain selected was that that resulted for maximum counting efficiency and minimum background.

It is necessary to achieve low background counting rates to reach the MDA values required by the proposed method. The background reduction means employed by the Quantulus include both passive shielding and an active guard detector. The passive shielding is graded, comprising 630 kg of lead in an asymmetric shape (20 cm thick over the counting chamber), a layer of cadmium shielding, and an internal copper canister shield which contains the active guard. The active guard detector is a canister surrounding the counting chamber that contains a liquid scintillation fluid. Two photomultiplier tubes (pmts) are used to detect scintillation in the active guard detector. Their output is compared to the output from the pmts registering sample scintillation. If a time interval of <15 ns separates pulses from the two sources, the data pulse is vetoed. By such means backgrounds of 0.7–1.1 cpm are routinely measured with counting efficiencies of approximately 40% for a 7 mL sample comprising equal volumes of water and the commercial scintillation cocktail UltimaGold LLT.

The tritium enrichment was the result of alkaline electrolysis using laboratory-built apparatus similar to that described in Östland and Werner (1962). The benzaldehyde was burned quantitatively in a 300 psig pure oxygen atmosphere using a Parr bomb combustion apparatus, 8–10 g at a time until approximately 40 mL of water was accumulated.

Reagents. The benzaldehyde samples were selected from samples received at the Center for Applied Isotope Studies for stable isotope and ^{14}C analysis. Deuterium measurements confirmed the authenticity of both the "natural" and the petroleum-derived samples, where for Ben 864, $\delta\text{D}(0/00) = -102$, indicating a natural origin, and for Ben 865, $\delta\text{D}(0/00) = 378$, indicating a petroleum origin. The deuterium concentrations are relative to standard mean ocean water (SMOW), which establishes zero on the δD scale, and were measured with a Finnigan-MATS isotope ratio mass spectrometer, Model 151. Additional confirmation derived from $\delta^{13}\text{C}$ (0/00) measurements and carbon dating. The $\delta^{13}\text{C}$ (0/00) measurements were relative to the CO_2 gas obtained by reacting the Cretaceous belemnite, *Belemnitilla americanata*, from the Peedee Formation of South Carolina with 100% phosphoric acid, which establishes zero on the $\delta^{13}\text{C}$ scale, abbreviated PDB. The measurements were performed with a Finnigan-MATS isotope ratio mass spectrometer, Model 151, for both the natural and petroleum-derived samples. For the natural sample $\delta^{13}\text{C}$ (0/00) = -29.3 (SD = 0.7) and for the petroleum-derived sample $\delta^{13}\text{C}$ (0/00) = -26.3 (SD = 0.6). Carbon dating was based on converting the sample to water and carbon dioxide. The water was used for the tritium analysis discussed in this paper and the CO_2 for benzene synthesis. The benzene thus formed was

counted in a LSC. The results showed that the natural sample was modern, or 16.3 ± 0.04 dpm/g of carbon, and the synthetic sample also modern, or 16.8 ± 0.4 dpm/g of carbon. We knew therefore that the ^{14}C concentration in the petroleum-derived benzaldehyde had been manipulated on the basis of the deuterium and the $\delta^{13}\text{C}$ measurements. Thus, the origin of the benzaldehyde samples used in this study was supported by three independent analytical techniques, each employing NIST traceable standards (Culp and Noakes, 1990).

Each enriched sample was mixed with 7 mL of ULTIMA GOLD LLT scintillation cocktail, manufactured by Packard Instrument Co. Prior to electrolysis each water sample was made alkaline (0.312 M) with 8.72 M NaOH made with 68 g of dry sodium peroxide and 200 g of "dead" water. "Dead" water refers to water known to be free of tritium. The dead water used in this study was obtained from a deep, frequently pumped well in a Cretaceous aquifer near Aiken, SC. Its tritium activity level was reported to be 0.065 ± 0.130 pCi/L (1 σ) (Tritium Laboratory, University of Miami). The same dead water was used as the background for the experiments.

Procedure. Two benzaldehyde samples were selected, the first known to be a plant extract and the other known to be petroleum-derived and adulterated with ^{14}C , and were quantitatively (>99%) combusted in pure oxygen, yielding water and carbon dioxide.

Each water sample was made alkaline prior to electrolysis. After 4 days of attended electrolytic tritium enrichment, the sample volumes had diminished to about 10% of the starting volumes (Bogen, 1990). The tritium enrichment factors were determined on the basis of deuterium measurements before and after electrolysis, the sample volume reduction, and $\dot{\eta}$, the electrode characteristic separation factor (Takahashi, 1968). Each sample and background standard was combined with the scintillation cocktail and counted in a LSC for 100 min (live time) 28 times. The error in each of the 28 counts was considered to be normally distributed, and the "z" scores were computed. The region of significance was selected to be included by $z = \pm 1.96$. The sample and background counting data that passed the significance test were pooled and used to determine the minimum detectable activity (MDA) for the tritium.

RESULTS AND DISCUSSION

The method depends on the notion that if an organic chemical is extracted from a botanical grown in an environment with a given level of tritium in surface water, then the extract will exhibit a corresponding tritium concentration, diminished by tritium decay during the time after the sample was taken and isolated to the time of the measurement. However, the tritium activity in a petroleum-derived organic chemical should be very low because of its long sequestration, during which the hydrocarbon-bound tritium atoms decay without replenishment. (Isolation for 100 years, or about 8 tritium half-lives, results in the decay of 99.62% of the tritium.) During and after synthesis based on petroleum-derived hydrocarbons, some exchange between labile protons and tritons may occur opportunistically but to no more than a fraction of natural levels (Stewart, 1967; Streitwieser and Heathcock, 1981). A statistical condition of unambiguously measuring the tritium concentration in the presence of background is that the magnitude of the tritium measurement exceed that of the background by a factor of ≥ 2 . The selection of the factor 2, while arbitrary, is a customary signal-to-noise ratio for many counting experiments (U.S. Environmental Protection Agency, 1980). Therefore, identification of a natural or a petroleum-derived product by tritium concentration is subject to the same signal-to-noise ratio.

Because the tritium level found in natural products is low, without concentrating tritium or enrichment,

Table 1. Results from Combustion of Benzaldehyde Samples

	extract oxidized (mL)	water yield (mL)	fraction oxidized to water
Ben 864 (N)	75	37.9	0.992
Ben 865 (S)	82	41.6	0.996

Table 2. Data Used for Calculation of Tritium Enrichment Factor^a

	D_i (ppm) start	D_f (ppm) end	α	initial vol V_i	final vol V_f	V_i/V_f	β	T_f/T_i
Ben 864 (N)	140	1265	9.534	37.89	3.24	11.69	13.348	9.727
Ben 865 (S)	215	2727	52.86	41.56	3.12	13.32	74.002	12.862

^a Values for the concentration of deuterium before and after enrichment, D_i and D_f , are measured by isotope mass spectrometry. Volumes of water before and after enrichment, V_i and V_f in mL, weighed to the nearest 0.01 g. The deuterium separation factor α is calculated based on the deuterium ratio and the volume ratio using eq 1. The tritium separation factor β is calculated using eq 3. The ratio of the concentration of tritium after and before enrichment, T_f/T_i , is calculated using eq 2.

analysis of the tritium would not be possible. Tritium enrichment is most conveniently conducted in an aqueous medium; consequently, the organic extract is quantitatively oxidized to water and CO₂. The water thus formed is tritium enriched.

Benzaldehyde was selected as the model compound for this work because both well characterized "petroleum-derived" and "natural" examples (Culp et al., 1990) were available and also because of its importance as a flavorant. All of the tritium, deuterium, and hydrogen from the quantitative oxidation of benzaldehyde is incorporated into water. The results of the combustion of the benzaldehyde samples are shown in Table 1.

Method Calibration. The tritium enrichment technique must be carefully calibrated for quantitative results. The calibration method relies on the determination of the ratio, $\hat{\eta}$, of the logarithms of the tritium separation factor and the deuterium separation factor shown in eq 3. The magnitude of the separation factors varies according to the composition of the electrodes. The electrode materials used for this experiment were iron for the cathode and nickel for the anode, which gave $\hat{\eta} = 1.43 \pm 0.08$.

Using the data presented in Table 2, eqs 1–3 were used to calculate the tritium enrichment factor, T_f/T_i . The basis for their use was established in 1971 by Östland and Werner (1962).

$$\log(V_i/V_f) = [\alpha/(\alpha - 1)] \log(D_f/D_i) \quad (1)$$

$$\log(V_i/V_f) = [\beta/(\beta - 1)] \log(T_f/T_i) \quad (2)$$

V_i is the initial sample volume, V_f is the final sample volume, D_i is the initial deuterium concentration, D_f is the final deuterium concentration, T_i is the initial tritium concentration, T_f is the final tritium concentration, α is the separation factor for deuterium, β is the separation factor for tritium, and $\hat{\eta}$ is a constant depending on the electrode materials.

$$\log \beta / \log \alpha = \eta = 1.43 \pm 0.08 \quad (3)$$

Thus, measuring the concentration of the deuterium and the sample volume before and after electrolysis provides sufficient data to calculate the tritium enrichment factor once the value for $\hat{\eta}$ is measured using tritium standards (Östland and Werner, 1962).

Method Results. The samples designated Ben 864 and Ben 865 were counted with three different counting time intervals, referred to as runs 1, 2, and 3. The principal parameter values and the results of the runs, with the counting error of each calculated to a 95% confidence level, are presented in Table 3. For each of the runs the activities are given as the average of the 28 measurements, computed in terms of the tritium activity of both the product combusted and the water of combustion from each sample.

The data labeled runs 1–3 in Table 3 are the results from three counting experiments conducted for the three different counting time intervals, ranging from 1000 to 2800 min. The enrichment factors differed substantially between Ben 864 and Ben 865 (9.727 vs 12.862); however, the backgrounds and the counting efficiencies from run to run differed only negligibly. The effect of the difference in the enrichment factors is apparent from the MDA value differences. If the enrichment factors for Ben 864 and Ben 865 had been the same, the MDA for each run in the Ben 864 series would have been the same as that for the corresponding run from the Ben 865 series.

A comparison of the MDA and the activity values calculated from the data raises a question of validity for one result. The conditions for run 3 for the Ben 865 series predict a MDA of 4.42 pCi/L and the activity measured is less, 3.50 pCi/L. Comparison of the counting times and the MDA indicates that with increased counting time the MDA diminishes; therefore, an inadequate counting time was used for run 3 in the Ben 865 series, rendering the calculated value of 3.50 pCi/L indistinguishable over the MDA of 4.42 pCi/L. The analysis is not necessarily defeated by such a result as long as the value of 4.42 pCi/L is used in the determination of the nature of the sample, i.e. natural vs petroleum derived.

Generally it is desirable for the calculated activity to be more than the MDA. The results and data for runs 1 and 2 exhibit the more desirable relationship because the counting time intervals employed are longer. Run 1 exhibits the lowest MDA of all of the runs and the longest counting time interval. Because the improvement in the MDA goes as the square root of the factor by which the counting time interval is increased, it is clear that there is a practical constraint on increasing the counting time interval indefinitely.

The other parameter that can be varied to improve the MDA is the enrichment factor. As the enrichment factor is increased the MDA decreases. The lower MDA for the Ben 865 runs, compared with those from Ben 864 series of runs, is the result of an enrichment factor for Ben 865 that is larger (12.862) than that for Ben 864 (9.747). The penalty for increasing the enrichment factor is the necessity to electrolyze more water and therefore oxidize more product.

The data indicate a clear difference between the samples: 14.34 ± 2.84 pCi/L for the water of combustion from the natural product and 6.40 ± 1.97 pCi/L for that from the petroleum-derived product. The activities measured for the natural and petroleum-derived samples are more than twice the background sample. According to the selection criteria given for significance, the natural product is statistically unmistakable. Additionally, both are distinct from background. The natural result is also in good agreement with the average steady-state concentration of tritium, 14.4 pCi/L, given for the northern hemisphere (Okai et al., 1994).

Table 3. Counting Results for Combusted Product and Water of Combustion

sample/run	count time (min)	counting efficiency	activity product (pCi/L)	activity water (pCi/L)	% error (1.96 σ)	MDA product (pCi/L)	MDA water (pCi/L)
Ben 864							
run 1	2763.27	0.423	6.81 \pm 1.17	13.48 \pm 2.33	17.25	1.84	3.65
run 2	1381.60	0.444	7.68 \pm 1.68	15.19 \pm 3.34	21.95	2.63	5.20
run 3	986.89	0.442	6.44 \pm 1.98	12.74 \pm 3.92	30.79	3.13	6.20
Ben 865							
run 1	2763.27	0.423	2.78 \pm 0.82	5.49 \pm 1.61	29.38	1.31	2.60
run 2	1381.60	0.444	3.70 \pm 1.18	7.31 \pm 2.33	31.83	1.87	3.71
run 3	986.89	0.442	1.78 \pm 1.37	3.50 \pm 2.70	77.01	2.23	4.42

Method Optimization. Because of the expense and limited amounts available of the botanical sample, it is worth considering the lower limit on the amount of extract that is required to authenticate a given material according to this method. Ultimately, the signal-to-noise ratio will govern the lower limit of resolution of any such analysis. For counting experiments, the signal-to-noise ratio is often defined as twice background (noise). The magnitude of the difference between measurements that can be regarded as statistically distinct depends on the extent of the overlap of their error distributions.

The overall sensitivity of the analysis is based on the parameters that controlled the counter and the amount of sample analyzed. For any counting experiment, the background and the counting parameters determine the lowest level of detection and therefore define the practical analytical limitations of the method, that limitation being the counts per minute that are equivalent to background, referred to as the minimum detectable activity (MDA) when scaled by sample mass or volume and counting efficiency [U.S. Environmental Protection Agency, 1980; U.S. Department of Energy, 1990; CFR 141.25(c)]. The MDA is expressed in units of picocuries per unit mass or volume, pCi/L for this study. The MDA, defined as the activity that exceeds background activity by a factor of 2, 95% of the time, is calculated according to eq 4 (U.S. Environmental Protection Agency, 1980).

Minimizing the MDA will define the magnitude of the statistically significant (minimum) difference between (a) the background and (b) the signal plus background that is statistically significant. Equation 4 shows that the three parameters that can be manipulated are counting time, background, and sample volume. The counting time can be increased indefinitely to improve the MDA; however, there are practical limitations as to how long a counting experiment should be conducted. They include long-term nonrandom variations in the external background, which can degrade counting experiments that are conducted for very long times.

$$\text{MDA (pCi/L)} = (4.66\sqrt{B/T})/2.22EVD \quad (4)$$

In eq 4 B is the background rate (cpm), T is the counting time (min), E is the counting efficiency, V is the volume of the sample (L), $2.22 = \text{dpm/pCi}$, λ is the decay rate constant for tritium = $1.0602 \times 10^{-7} \text{ min}^{-1}$, t is the elapsed time during which tritium has decayed, and D is tritium decay = $e^{-\lambda t}$.

It is clear from eq 4 that increasing the counting time reduces the MDA by the square root of the reciprocal of the factor by which it is increased. Increasing the volume reduces the MDA by the reciprocal of the factor by which it is increased. Clearly, increasing the volume has a greater effect in lowering the MDA than increasing the counting time.

Table 4. Sample Size Minimums for Selected Extracts

	chemical formula	molecular weight	amount equivalent to 1.93 M hydrogen (g)
benzaldehyde	C ₇ H ₆ O	106.12	68.27
cinnamaldehyde	C ₉ H ₈ O	132.15	63.76
anethole	C ₁₀ H ₁₂ O	148.20	47.67
vanillin	C ₈ H ₈ O ₃	152.14	73.41

The effective volume of sample counted increases as the concentration of tritium is increased; thus, the MDA is reduced. Electrolytic tritium enrichment favors the electrolysis of untritiated water because the ratio of the concentration of protons to tritons is a whole number multiple of 1×10^{18} ; thus, tritium is concentrated. For the subject work, electrolytic tritium enrichment was employed to concentrate the tritium, thus decreasing the MDA by effectively increasing the volume of the sample counted. The method and apparatus employed for the electrolysis of water are described by Östland and Werner (1962).

A reasonable counting time limit is 2880 min or 2 days. The normal background range will be between 0.7 and 1.1 cpm. The counting efficiency for a 7 mL counting vial and a 3 mL aqueous sample will be slightly >40%.

Rearrangement of eq 4 with the sample volume, V , the independent variable gives

$$V = [(4.66\sqrt{B/T})/2.22E]/\text{MDA} \quad (5)$$

Using the values for the parameters given above, the ideal minimum volume of water sample counted as a function of MDA is given by

$$V = 0.09277/\text{MDA} \quad (6)$$

The activity selected for the MDA should be as large as can be tolerated and still allow signal over background to be statistically resolved. Selecting the activity equivalent to background as the MDA provides a 50% chance that a net activity equivalent to background will be statistically significant (e.g. not an estimate of background). Thus, for an MDA of 6.24 pCi/L, the minimum burn-derived water sample volume, V , is equal to 14.5 mL or 0.805 mol of water or 1.610 mol of hydrogen. To measure an activity level equal to the MDA ensures that the risk (probability) of falsely authenticating a sample is 0.50. This argument depends on the assumption that no tritiated water is lost during electrolysis. However, up to 20% of the tritiated water may be lost during electrolysis and sample transfer, although less is usual. Correcting for the loss results in a 25% increase in the water volume required for enrichment or 17.4 mL or 1.93 mol of hydrogen.

Table 4 lists the minimum amount of plant extract, in grams, required for combustion and analysis for authentication.

The proposed method is subject to certain limitations. It is unlikely that a synthetic product derived from starting materials that are of plant/botanical origin could be distinguished from an authentic natural product by any technique currently available. However, adulteration of a petroleum-derived product with tritium may be detected in many cases. Tritium adulteration is particularly demanding due to its relatively high specific activity and hence low concentration of the isotope.

At tritium levels found in the natural benzaldehyde used for this study, only 1 proton site in $\sim 6 \times 10^{19}$ was occupied by a triton. For the amount of natural product used, the one labile proton site per molecule provided $\sim 4.5 \times 10^{23}$ labile sites. Thus, if exchange were the adulteration strategy and only labile sites occupied by tritium, then only ~ 7550 labile sites of the $\sim 4.5 \times 10^{23}$ available would be occupied by a triton. To form such a product would be demanding. For example, if the measurement of an alleged natural product resulted in a tritium concentration that exceeded the natural levels, it should be concluded that the sample was adulterated. Even if the tritium concentration lay between the background and ambient ground water tritium levels, it would be prudent to consider the sample adulterated until other analytical methods proved otherwise (i.e. carbon dating, $\delta^{13}\text{C}$, and deuterium measurements).

The alternative adulteration strategy of synthesizing a product from wood lignin from trees that were 36 years old, for example, would result in a product having tritium levels about one-eighth that found in a natural product, an ample difference for determining a synthetic product by the proposed analysis. (Note 36 years is approximately 3 half-lives of tritium.) Thus, using another natural product as the starting material for synthesis of another product confers no particular advantage if it is older than a few months. The fact that tritium half-life is short is an advantage to the proposed method of analysis/authentication, but the longer half-life of radiocarbon renders its use for this purpose quite insensitive.

Another strategy for adulteration could be based on dilution, that is, adding enough tritium-enriched product, albeit a relatively small amount, to a larger quantity of petroleum-derived product so that the resulting tritium levels resemble natural levels. With respect to tritium analysis, such a strategy would defeat the ability of the analysis to authenticate the alleged natural product. However, it would be a daunting task to simultaneously adjust radiocarbon and the deuterium concentrations and thus defeat the other techniques for validating natural products.

More sophisticated strategies for adulteration can be developed no doubt, not, however, without increased expense. As better sensitivities for the method are achieved and more precise measurements made, adulteration strategies will become less effective in producing a synthetic product that can pass as natural.

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