

FOREWORD

Marine contamination by petroleum, whether by natural seepage or by spills from ships at sea, by accidents in harbour or at offshore installations or by atmospheric or terrigenous input is by no means a new or rare phenomenon. In recent years however, the problems have been highlighted not only by the increased utilisation and marine transport of oil but also by a number of spectacular accidents which have raised questions about possible effects on the ecosystem. A number of detailed studies have been carried out in an attempt to answer these questions. The demands for such knowledge have been further increased by the various questions raised as a result of expansion of offshore exploration and exploitation for oil, particularly in environments hostile to these operations, in regions as far apart as the northern North Sea and the coast of Alaska.

Consequently, diverse aspects of the problem are being studied in several parts of the world by chemists and biologists who are often asking the same questions but using different approaches and sometimes producing conflicting views. Against this background, it seemed timely therefore to bring together a group of scientists from university, industry and government, actively engaged in such work, to examine and discuss common problems relevant to petroleum hydrocarbon contamination of the marine ecosystem and so a Work-

shop was sponsored by the International Council for the Exploration of the Sea, and held in Scotland at Aberdeen in September 1975.

The Workshop considered methodology, occurrence and fate in the environment, and effects on the ecosystem of petroleum hydrocarbons in the sea. Most of the papers presented and updated where necessary, are brought together in the present volume together with an edited version of the recorded discussion that followed each session. Of necessity, the reportage of the discussion is very brief although the proportion of time available for discussion compared favourably with that set aside for formal presentation of the papers. In preparing the discussion reports, the editors were assisted in particular by Dr R. Hardy, Dr R. Johnston, Mr P. R. Mackie and Dr I. C. White, and by comments from several contributors.

No attempt was made to produce specific recommendations but a study of the papers in this volume does give a clear indication of several lines of research which must be followed up before an adequate understanding can be reached of the effects of petroleum in the sea and it is evident that widespread monitoring operations will be fully effective only when the basis of our knowledge has been thus extended.

A list of participants to the workshop may be found in Appendix I.

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THE ULTRASONIC DISPERSION, SOURCE IDENTIFICATION, AND QUANTITATIVE ANALYSIS OF PETROLEUM OILS IN WATER

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We describe three current projects involving the development of methods for the quantification and source identification of water dispersed oils, and for the preparation of stable oil-in-water dispersions. Adaptations of existing solvent extraction, IR quantification, and GC identification methods are discussed, and some newly developed techniques are presented. The techniques include: 1) a method for inducing comparable volatility losses in milligram and sub-milligram amounts of oils, and thereby enhancing their source identification; 2) a rapid adsorption method for separating hydrocarbons from other organics, and thereby achieving more selective petroleum oil quantification; 3) a method for using an ultrasonic device to prepare stable oil-in-water dispersions, having a known oil content.

INTRODUCTION

Petroleum oils enter the water column of the aqueous environment through many pathways. Offshore drilling operations, ships' cleaning operations, damaged tankers and storage tanks, industrial and municipal outfalls, and natural seeps all contribute their share. Discharged petroleum oils thereby constitute a major pollution problem, that is under intensive evaluation by the U.S. Environmental Protection Agency (EPA), other government agencies, various industrial organizations, and by segments of the academic community.

Most standardized laboratory methods that are currently used to monitor oil pollution are procedures for identifying the point of discharge of visible oil slicks and shoreline residues, and methods for quantifying the total extractable organics in water. Few corresponding methods exist for identifying the source of sub-milligram quantities of water dispersed oils, and for estimating the petroleum oil content of waters that also contain substantial amounts of other extractable organics.

Our paper addresses these needs. We describe two current projects to develop methods for the quantification and source identification of water dispersed petroleum oils, and a procedure for preparing stable oil-in-water dispersions, having known oil content. These projects are still underway, and our conclusions are therefore tentative. We hope to publish final results in the near future.

OIL IDENTIFICATION

Several oil identification methods were recently published by the American Society for Testing and Materials (ASTM). They include a gas chromatographic (GC)

procedure (ASTM D3328-74T), and a method for using nickel, vanadium, sulfur, and nitrogen components of oil for the identification of oil (ASTM D3327-74T). Other recently published, and soon to be published ASTM methods are also relevant. They include a sample preservation technique (ASTM D3325-74T), a sample preparation method (ASTM D3326-74T), an infrared (IR) procedure (provisional ASTM designation D3414-75T), and a data handling technique (provisional ASTM designation D3415-75T). When properly combined, these methods can be used to identify the source of discharge of oil pollutants, i.e., to "fingerprint" oils.

While these ASTM methods emphasize the identification (fingerprinting) of oil pollutants that occur as visible surface slicks and shoreline residues, our present interest as described in this paper, is the development of a method for fingerprinting much smaller quantities of oils, i.e., oils that are extracted from the water column. We decided to utilize and integrate existing procedures wherever possible. Consequently, ASTM D3328 was selected for matching oils by GC. Similarly, existing procedures were used for solvent extraction (Gruenfeld, 1973) and IR quantification (Gruenfeld, 1975). The need for quantification prior to identification is discussed below.

Optimum intercomparison of oils is enhanced by stripping volatile components from weathered and unweathered oils, to yield residues with similar volatility losses. ASTM D3326 achieves this by distillation: 50 ml portions of slicks, shoreline residues, and unweathered oils are heated to yield 280°C+ distillation residues that are then matched by GC, IR, and the elemental

analysis methods. But, the requirement for 50 ml quantities of oil greatly exceeds the amount of oil that is usually available from the water column; i.e., water samples normally yield only microlitre (mg) quantities of dispersed oil. Therefore, we developed a procedure for treating 0.5 and 30 mg quantities of oil to yield residues with GC profiles that closely resemble the residue profiles of ASTM D3326.

It should be noted that other methods are available for GC identification of water dispersed oils (CONCAWE, 1972; Dell'Acqua et al., 1975). But these procedures do not attempt to optimize intercomparison of oils by yielding residues with similar volatility losses, nor do they benefit from the incorporation of a standardized ASTM method.

OIL QUANTIFICATION

Many methods are available for estimating the amount of oil in water. Some measure extractable aromatics by UV absorption or fluorescence techniques (Zsolnay, 1973; Levy, 1971; Gordon and Keizer, 1974). Others measure extractable hydrocarbons by IR, following Florisil or silica gel adsorption chromatography (CONCAWE, 1972; Brown et al., 1974; Yu and Coleman, 1975), while still others measure total extractable organics by IR and gravimetric procedures (EPA, 1974; Gruenfeld, 1975). All of these methods can estimate $\mu\text{g/ml}$ levels of oil in water, but only the UV absorption and fluorescence methods, and one IR procedure (Brown et al., 1974) estimate 1–10 $\mu\text{g/litre}$ levels. This IR method is somewhat cumbersome, however, because exceedingly large water samples are needed (usually 20 litre), while the UV absorption and fluorescence methods suffer from substantial variability in response from oil to oil, thereby handicapping selection of meaningful reference standards.

Our current interest as described in this paper, is the development of a method for estimating the dispersed petroleum oil content in water columns near surface slicks, ships' ballast water discharges, offshore waste disposal sites, and offshore platforms *inter alia*. The method should be capable of distinguishing petroleum oils from other extractable organics, and be appropriate for estimating oil in water concentrations below 10 $\mu\text{g/litre}$. We are evaluating, as the method of choice, a rapid silica gel adsorption technique (Longbottom, 1974), followed by IR measurement of hydrocarbons. The work is still incomplete, however, and consequently discussion is limited to evaluation of the silica gel adsorption step.

OIL DISPERSION

Adequate methods are needed for preparing stable oil-in-water dispersions that can be further diluted with

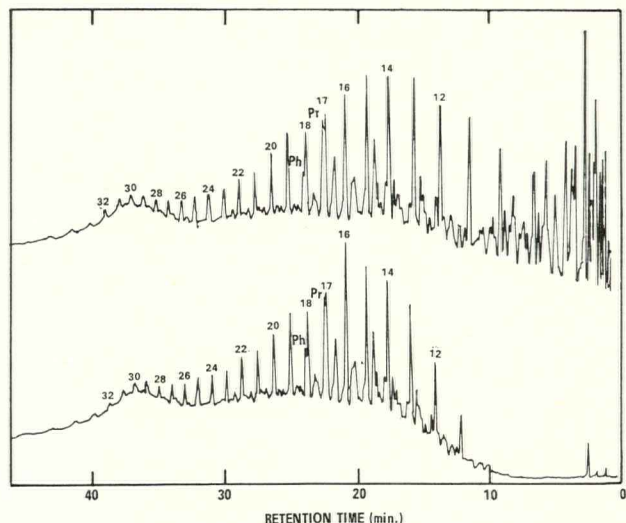


Figure 21. South Louisiana Crude oil. Untreated neat oil (upper chromatogram). After ASTM D3326 distillation (lower chromatogram). Numbers refer to *n*-alkanes. Pr and Ph refer to pristane and phytane, respectively.

water. Use of stable dispersions having known oil content can enhance bioassay tests of oil toxicity, and performance evaluations of oil quantification methodology. Most dispersions are now prepared by vigorously shaking or mechanically mixing oils with water, but separation of oils can quickly ensue. Consequently, we evaluated the use of a commercially available ultrasonification device for preparing stable dispersions. We then examined the resulting dispersions for stability and miscibility with water. We also tested the ultrasonically dispersed oils for spectral and chromatographic alterations that may interfere with their quantification and identification.

EXPERIMENTAL

APPARATUS

Gas chromatographic determinations were performed with a Perkin-Elmer Model 900 GC instrument¹, equipped with a flame ionization detector. A 3 m by 3 mm O.D. (0.6 mm wall) stainless steel column was used, packed with 10% OV-101 on 60/80 Chromosorb W (AW-DMCS treated). Helium at 40 ml/min was the carrier gas. Temperature programme: initial-50°C for 2 min., final-325°C, programme rate-8°C/min., injector-300°C.

Infrared spectroscopic determinations were performed with a Perkin-Elmer Model 457A IR instrument. Absorbances were measured in 10 mm rectangular silica

¹ Mention of trade names or commercial products does not constitute endorsement by the U.S. Government.

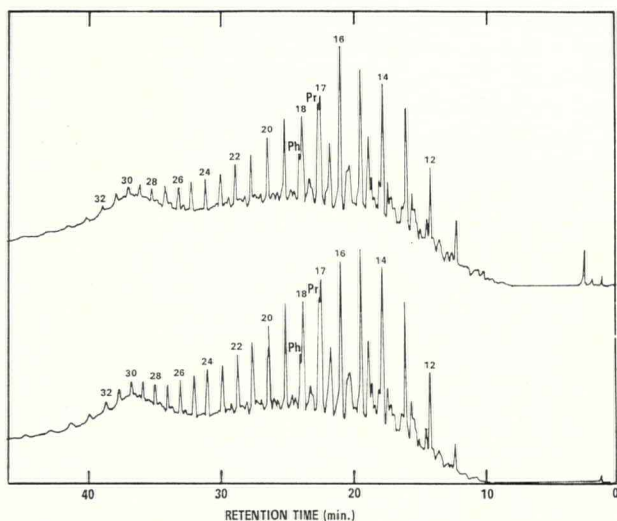


Figure 22. South Louisiana Crude oil. After ASTM D3326 distillation (upper chromatogram). After the 70 mg neat oil treatment procedure (lower chromatogram). See legend of Figure 21 for key to numbers and letters.

cells (Beckman Instruments Incorporated, catalogue number 580015), using Perkin-Elmer cell holders (catalogue number 186-0091).

Ultrasonic dispersions were prepared with a Branson Sonic Power Company (Danbury, Connecticut, USA) model W185 Sonifier Cell Disruptor, using a 1/2 inch disruptor horn with plain conical tip.

REAGENTS

Spectroanalyzed carbon tetrachloride (Fisher Scientific Company, Catalogue Number C-199), and Freon 113 solvent (E. I. Du Pont De Nemours and Company, Inc.) were used. Freon 113 is a Du Pont designation for 1,1,2-trichloro-1,2,2-trifluoroethane, and this reagent is also available from other manufacturers under various trade names.

Separation of hydrocarbons from other organics was accomplished with Davidson Type 923 silica gel (100–200 mesh).

PROCEDURE

Oil identification

Simulated oil in water samples were prepared by ultrasonically dispersing a South Louisiana Crude oil in water. Sample extraction was achieved with four, 25 ml portions of Freon 113, following addition of acid and salt (Gruenfeld, 1973). Quantification of oils was accomplished by IR spectroscopy (Gruenfeld, 1975), followed by further dilution to yield final 100 ml Freon solutions containing 0.5 or 30 mg oil. Simulation of the ASTM D3326 distillation was accomplished by strip-

ping the solutions to final 1–2 ml volumes, in 150 ml beakers, with the aid of a steam table and a filtered air stream and the concentrates were transferred to 10 by 30 mm glass vials. The vials containing 30 mg portions of oil were suspended in a 40°C water bath, and a filtered air stream was used to remove final solvent traces. This condition was maintained for 10 additional minutes. The vials containing 0.5 mg portions of oil were maintained at room temperature, the air flow was turned off just before total solvent removal and final solvent evaporation occurred spontaneously. This condition was maintained for 10 additional minutes. Distilled solvent was used for the lower concentration determinations. Small amounts of CCl_4 (10–20 μ l) were then added to each vial for GC injection.

In order to enhance intercomparisons of neat oils with dispersed oils, the neat oils were treated as follows: vials containing 70 mg portions of neat (undispersed) oils were suspended in a 40°C water bath for 15 minutes, in the presence of a filtered air stream.

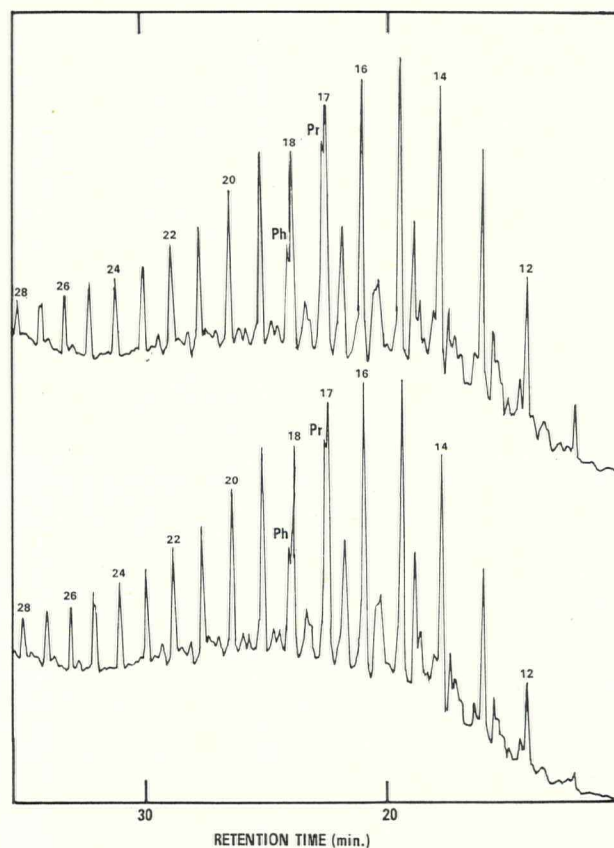


Figure 23. South Louisiana Crude oil. After the 70 mg neat oil treatment procedure (upper chromatogram). After the 30 mg dispersed oil treatment procedure (lower chromatogram). See legend of Figure 21 for key to numbers and letters.

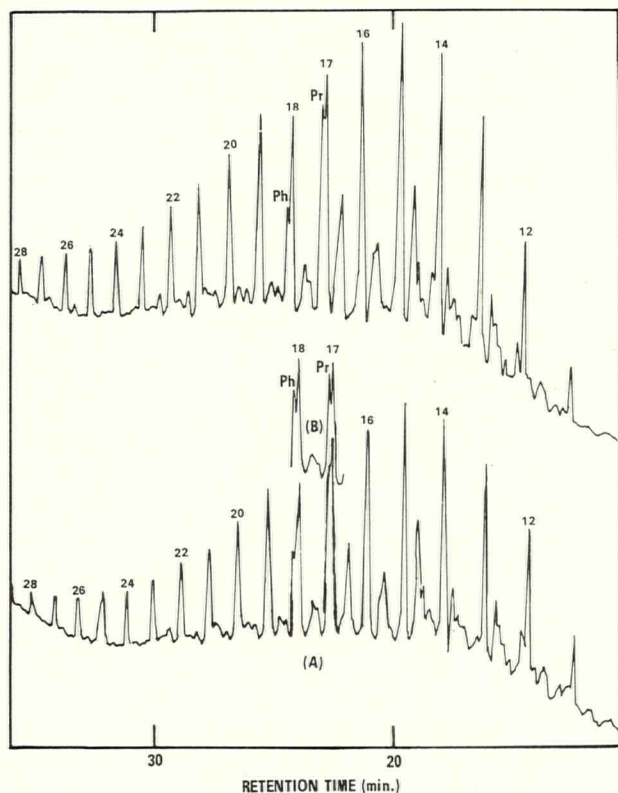


Figure 24. South Louisiana Crude oil. After the 70 mg neat oil treatment procedure (upper chromatogram). After the 0.5 mg dispersed oil treatment procedure (lower chromatogram): (A) - shows slight column degeneration, (B) - new column. See legend of Figure 21 for key to numbers and letters.

Portions of the residues were then injected onto the GC column.

Oil quantification

While modifications of existing procedures for oil extraction (Gruenfeld, 1973) and quantification (Gruenfeld, 1975) to improve sensitivity are planned, our present discussion deals only with a silica gel adsorption technique for separating petroleum oils from animal and vegetable oils, directly in CCl_4 solution. The latter oils contain many components that typify the water dispersed non-hydrocarbon organics that separate together with petroleum oils during solvent extraction. Carbon tetrachloride solutions of South Louisiana Crude and Number 2 fuel oils, and vegetable, olive, and cod liver oils were prepared in 100 ml volumetric flasks, at the following concentrations: petroleum oils - 20 mg/100 ml, non-petroleum oils - 100 mg/100 ml. These solutions were vigorously stirred for 5 and 10 minute intervals with a magnetic stirrer, after additions of 3 g activated and partly deactivated silica gel. Activation was achieved by maintaining 100 g portions of

silica gel at $150^\circ C$ for two hours. The degree of adsorption of the non-petroleum oils by silica gel, and the impact of silica gel adsorption on the petroleum oils were monitored by IR (Gruenfeld, 1975). The degree of oil removal from solution was compared graphically with the degree of silica gel deactivation and solution stirring time.

Oil dispersion

Ultrasonic dispersion was achieved by inserting the instrument probe into 50 ml graduate cylinders containing 50 ml water and accurately weighed amounts of oil. Maximum dispersing energy was applied for two minutes, while suspending the cylinders in an ice bath. Dispersions of a South Louisiana Crude oil (4.8 cSt at $38^\circ C$) and a Bachaquero Crude oil (1070 cSt at $38^\circ C$) were tested for stability and miscibility with water. Portions of the South Louisiana oil were also recovered after dispersion, and examined for ultrasonically induced chromatographic and spectral changes. Gas chromatography, and IR, UV, and fluorescence spectroscopy were used.

RESULTS AND DISCUSSION

OIL IDENTIFICATION

As previously explained, our immediate goal was to develop a procedure for enhancing comparisons of undispersed oils, with 0.5 and 30 mg quantities of water dispersed oils. We hoped to induce losses of volatiles in minute oil residues, that approximated losses induced by ASTM D3326.

The ASTM method strips off almost all components below the C_{11} normal alkane (Fig. 21). Our procedure for treating neat oils yields equivalent losses (Fig. 22). Similarly, our procedure for treating 0.5 and 30 mg portions of dispersed oils yields chromatograms that nearly match the chromatogram of the neat oil (Figs. 23 and 24). Therefore, within the constraints of our limited tests (one oil was used) it appears that our method can successfully correlate water dispersed oils with neat oils, for the purpose of source identification (fingerprinting). However, evaluation of the method with more oils is needed, especially following short term weathering in the water column. This work is now underway and will be reported in the near future. Meanwhile, our method offers a convenient technique whereby reproducible and intercomparable chromatograms of milligram and sub-milligram amounts of oil can be obtained.

OIL QUANTIFICATION

As part of our work to develop an appropriate oil quantification method, we evaluated a rather unique

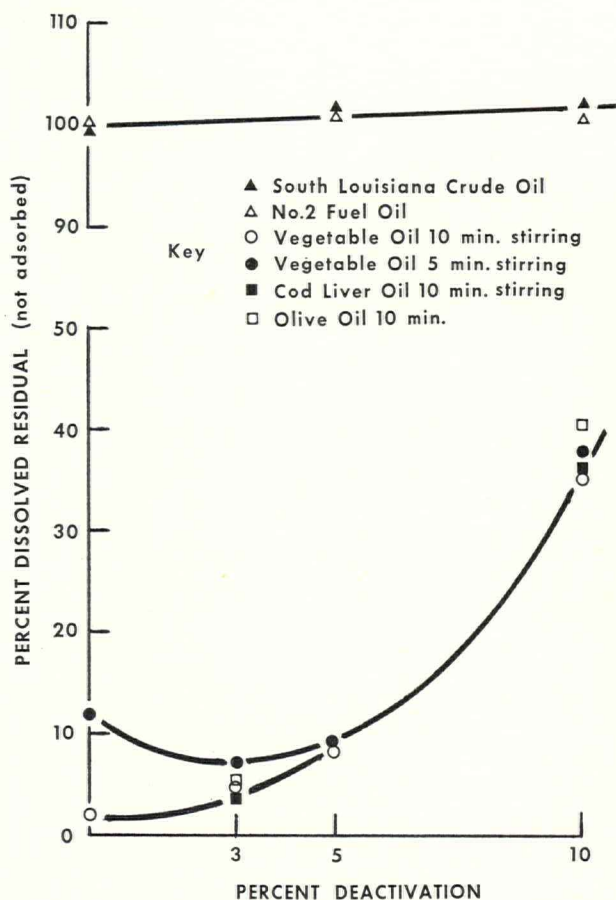


Figure 25. Adsorption of oils by silica gel in CCl_4 solution. IR measurements are at 2930 cm^{-1} , using 10 mm path length cells.

adsorption technique for separating petroleum oils from animal and vegetable oils. Separation was achieved by merely adding small amounts of silica gel to CCl_4 solutions of oils, and then stirring briefly. The non-petroleum oils were removed by silica gel, while the petroleum oils remained in solution. Consequently, we expect that this procedure will successfully separate most water dispersed petroleum oils from the non-hydrocarbon organics that are also extracted from the water column. It should be noted that Florisil can also be used in a similar manner (CONCAWE, 1972), but that silical gel is more effective (Longbottom, 1975).

In order to optimize the adsorption procedure, we evaluated the interaction of silica gel deactivation and solution stirring time, with oil separation efficiency (Fig. 25). Maximum separation of the petroleum oils from the non-petroleum oils was achieved with fully activated silica gel, and a 10 minute stirring period (stirring rate was not monitored). These conditions did not cause a visible loss of the petroleum oils. Increased silica gel deactivation, and reduced stirring time ad-

versely affected the method. The method appears quite practical for separating petroleum oils from non-hydrocarbon organics, prior to IR measurement. But, fully activated silica gel, and ample magnetic stirring (10–15 min.) are recommended.

OIL DISPERSION

Preparation of stable oil-in-water dispersions by ultrasonification offers some attractive possibilities. The concentrates are stable, infinitely miscible with water, and have a known oil content. They are useful for critical method evaluations, and can be added to water columns for various biological and toxicity tests. Our method was used recently to advantage in a study of the mode of accumulation of Number 2 fuel oil by the soft shell clam *Mya arenaria* (Stainken, 1975). Dispersion stability appears to diminish with increasing oil viscosity. The South Louisiana oil yielded 1% (10 000 $\mu\text{g/ml}$) dispersions that remained stable beyond four days. But, the Bachaquero oil yielded less concentrated dispersions, that were also less stable.

Since ultrasonification causes considerable heating resulting in oil vapourization losses, we incorporated an ice bath in the method to prevent these losses. Following ultrasonification of 6 and 30 mg portions of the two oils, recoveries in the range, 96–100%, were obtained. In addition, ultrasonically treated and untreated portions of the South Louisiana oil were compared by gas chromatography, and IR, UV absorption and fluorescence spectroscopy. No significant chromatographic or spectral changes were observed.

CONCLUSION

Current work to develop methods for the quantification and source identification of water dispersed petroleum oils, and for the preparation of stable oil-in-water dispersions, is discussed. Tentative procedures are presented for: 1) separating hydrocarbons from non-hydrocarbon organics for the purpose of oil quantification; 2) inducing comparable volatility losses in milligram quantities of oils to enhance their source identification; 3) preparing stable oil-in-water dispersions with known oil content, that are water miscible. Preliminary conclusions are provided regarding the successful application of the methods, but these conclusions are subject to future tests with more oils, and to integration of our procedures with already existing methods. Final results will be published in the near future.

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