# Determination of Mesityl Oxide and Diacetone Alcohol in Oilseed Meals and Flours[1]

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## ABSTRACT

Two methods were compared for quantitative determination of trace amounts of mesityl oxide and diacetone alcohol in acetone-extracted oilseed meals and flours. In the first, a simple, rapid and direct gas chromatographic procedure, a 0.04 g sample of oilseed meal or flour was placed between 2 small glass wool plugs in a liner of the injection port of a gas chromatograph. Water-saturated molecular sieve 5A (1.1 g) was added over the glass wool sandwich, and the liner was placed in the heated injection port and firmly secured. Mesityl oxide and diacetone alcohol, and other volatiles, were eluted rapidly from the sample onto the column by the combined action of heat, moisture, and carrier gas. The components then were resolved readily by temperature-programed gas chromatography on a 2 ft x 1/4 in. Porapak P column. The second method, which is time consuming and tedious, is a modification of the Todd azeotropic distillation procedure and was included, for comparison, as a conventional measure of the concentrations of mesityl oxide and diacetone alcohol. Samples of oilseed meals and flours were analyzed by both methods. The results are compared, and the relative merits of the two procedures are discussed.

[1]Presented at the AOCS Fall Meeting, Atlantic City, April 1971.
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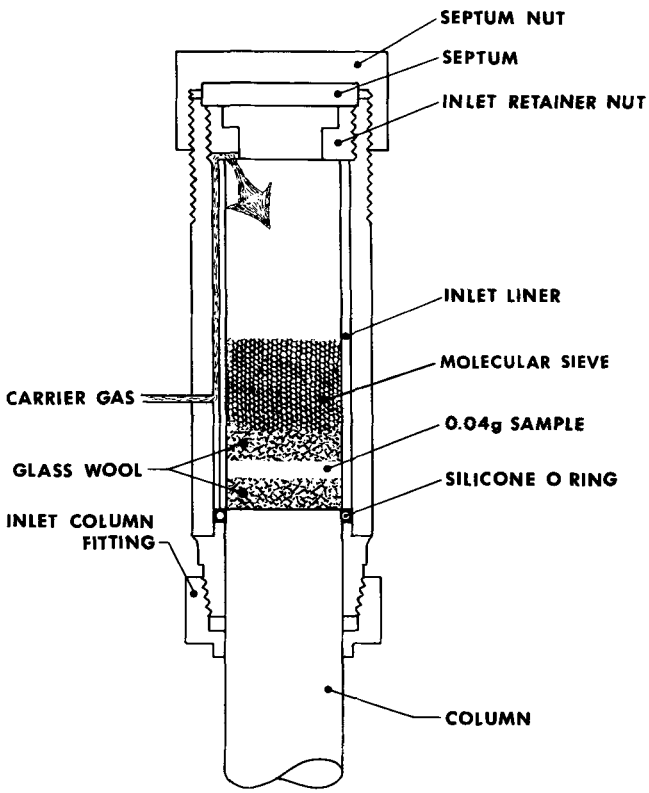


FIG. 1. Cross section of inlet of gas liquid chromatograph showing inlet liner with sample.

## INTRODUCTION

In recent years, acetone has been suggested as a processing solvent for certain oilseed applications. The azeotrope of acetone, hexane, and water, for example, has been reported to be an efficient solvent for extracting aflatoxins from mold-damaged oilseed meals (1). Also, aqueous acetone has been described as an effective means of removing gossypol from glanded cottonseed products (2). However, oilseed meals extracted with acetone sometimes have objectionable odors and flavors. Such off-flavors have been attributed to compounds formed by reaction of hydrogen sulfide with an acetone condensation product, mesityl oxide (3,4). Thus, a procedure is needed to detect and quantitate mesityl oxide and its precursor, diacetone alcohol, in acetone-extracted products. The volatilization procedures which were developed at this laboratory (5-7) for the determination of residual solvents in extracted meals are not suitable for the determination of mesityl oxide and diacetone alcohol, because these compounds undergo chemical changes during the long heating periods required for volatilization.

This paper reports the development of a rapid, direct gas liquid chromatographic (GLC) procedure (Procedure A) and a modification of the Todd azeotropic distillation procedure (8) (Procedure B) for quantitative determination of residual mesityl oxide and diacetone alcohol in oilseed meals and flours.

## EXPERIMENTAL PROCEDURES

### Materials

The following products were used: Porapak P, a porous polymer, from Waters Associates, Framingham, Mass.; ECNSS-S, a cyanosilicone-ethylene glycol succinate polymer, from Applied Science, State College, Pa.; molecular sieve 5A pellets, a synthetic zeolite (water saturated by exposing in thin layers to an atmosphere of 100% relative humidity for 24 hr); XE-60, a cyanosilicone; Chromosorb G, and silicone O-rings (preconditioned at 200 C for 2 hr to render them free of volatile impurities) from Teklab, Baton Rouge, La.; Pyrex glass wool manufactured by Corning Glass Works, Corning, N.Y.; and reagent grade diacetone alcohol and practical grade mesityl oxide from Eastman Kodak Co., Rochester, N.Y.

### Sample Preparation

*Direct GLC method*: A small plug of glass wool was placed in the end of the liner (3-5/16 in. length of 3/8 in. outside diameter borosilicate glass tubing) of the injection port of the gas chromatograph. The glass wool was tamped down lightly, and 0.04 g finely ground oilseed meal or flour was added on top of it. The sample was capped with another small plug of glass wool. Water-saturated molecular sieve 5A pellets, 1.1 g, were added over the glass wool sandwich of oilseed meal. The liner, containing the sample and molecular sieve, was inserted on top of the silicone O-ring in the preheated (100 C) injection port of the gas chromatograph. The inlet retainer nut then was tightened firmly to form a seal between the base of the inlet liner and the injection port. The septum was placed in position and tightened securely with the septum nut, thus forcing the carrier gas to flow upward and into the liner. Figure 1

illustrates the preparation of an inlet liner with sample and molecular sieve. Thus prepared, the sample was analyzed by direct GLC as described in Procedure A. At the conclusion of temperature program 3, the liner containing spent sample was removed from the gas chromatograph to allow the column to clean out during the final hold period.

*Azeotropic distillation*: The procedure used was that described by Todd (8), except for the following modifications: sample size was reduced from 50 to 25 g; 100 ml 20% sodium sulfate was used instead of 10 g sodium sulfate and 50 ml water; the mixture was stirred with a magnetic stirrer during distillation; and 0.5 µl aliquots of the distilled toluene fractions were analyzed by GLC as described.

### Correction Factor

Analysis for residual solvents by azeotropic distillation sometimes requires use of a recovery factor. To determine these factors for diacetone alcohol and mesityl oxide, the following procedure was used: Two (25 g) samples of cottonseed meal (determined to be free of mesityl oxide) were "spiked" with calculated quantities of mesityl oxide to provide one sample containing 25 ppm and another containing 100 ppm mesityl oxide. The same procedure was followed (using diacetone alcohol-free cottonseed meal) to obtain two cottonseed meal samples containing 25 ppm and 100 ppm diacetone alcohol. The respective meals then were analyzed by azeotropic distillation. Recoveries were essentially 100% for mesityl oxide at both levels and ca. 77% for diacetone alcohol at both levels. Therefore, only values for diacetone alcohol analysis by the distillation method were corrected.

### GLC Analysis

Operating conditions for the GLC analysis by direct GLC (Procedure A) and the toluene solution from azeotropic distillation (Procedure B) were as follows.

*Instrument*: Instruments used were Micro-Tek 2000 MF gas chromatograph with dual independent hydrogen flame detectors, Westronics LD 11 B recorder, and Infotronics CRS-100 integrator.

*Columns*: Columns were (A) 1/4 in. x 2 ft stainless steel U-tube, packed with 80-100 mesh Porapak P and (B) 1/4 in. x 6 ft stainless steel U-tube packed with 80-100 mesh Chromosorb G coated at a level of 5% with a 3 to 1 mixture, respectively, of XE-60 and ECNSS-S.

*Flow rates*: Flow rates were (A) helium 70 ml/min, hydrogen 60 ml/min, air 0.6 cu ft/hr (fuel and scavenger gas) and (B) helium 60 ml/min, hydrogen 55 ml/min, air 0.6 cu ft/hr (fuel and scavenger gas).

*Temperature*: Temperature was as follows for procedure A: initial hold 100 C for 7 min; program 1, 5 C/min for 10 min; program 2, 0.5 C/min for 2 min; program 3, 15 C/min for 3 min; final hold 200 C for 5 min; injector 105 C; detector 240 C. For Procedure B, temperature was: initial hold 40 C for 10 min; program 1, 2 C/min for 11 min; program 2, 7.5 C/min for 4 min; program 3, 0.5 C/min for 10 min; final hold 130 C for 11 min; injector 140 C; detector 190 C.

*Sample size*: Size of samples was (A) 0.04 g and (B) 0.5 µliter.

*Attenuation*: Attenuation was achieved with: (A) electrometer 10 x 1 and (B) electrometer $10^4$ x 1 until toluene peak emerged, then set to 10 x 1.

*Chart speed*: Speed for both (A) and (B) was 30 in/hr.

### Standardization

*Procedure A*: Six aqueous standard solutions were prepared and contained 0.6, 2, 12, 20, 40, and 60 mg mesityl oxide and diacetone alcohol/liter. The calculated volumes of mesityl oxide and diacetone alcohol were added from microsyringes of appropriate sizes to distilled water in either 1, 2, 5, or 10 ml volumetric flasks which then were

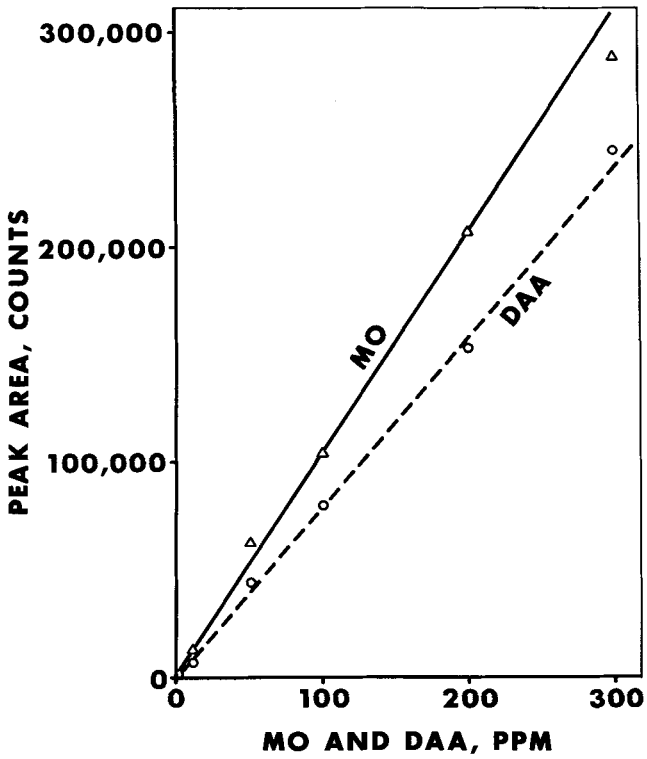


FIG. 2. Direct gas liquid chromatography calibration plots for converting peak areas to ppm of mesityl oxide (MO) and diacetone alcohol (DAA).

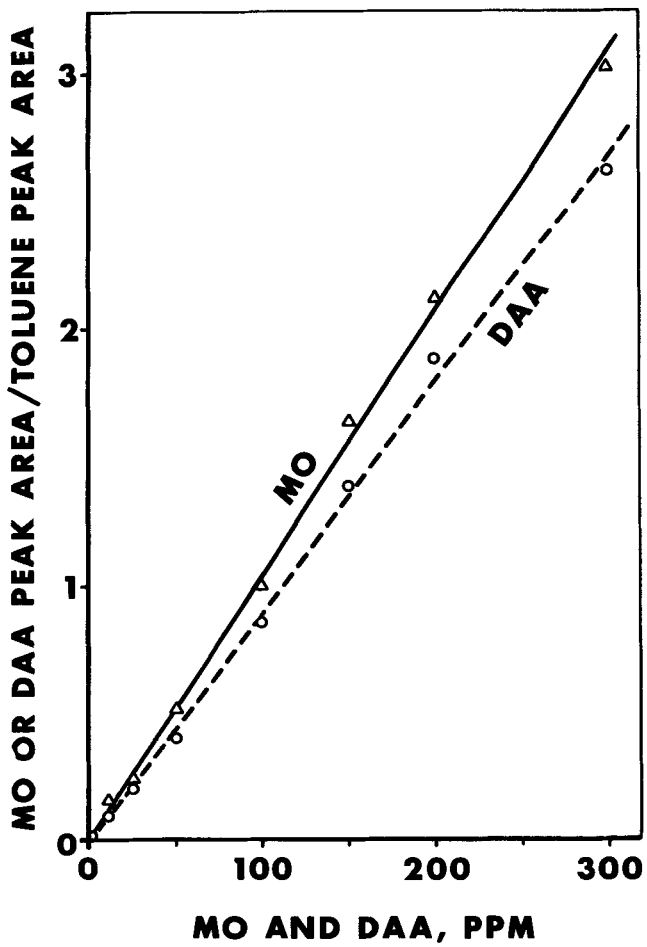


FIG. 3. Azeotropic distillation calibration plots for converting peak area ratios to ppm of mesityl oxide (MO) and diacetone alcohol (DAA).

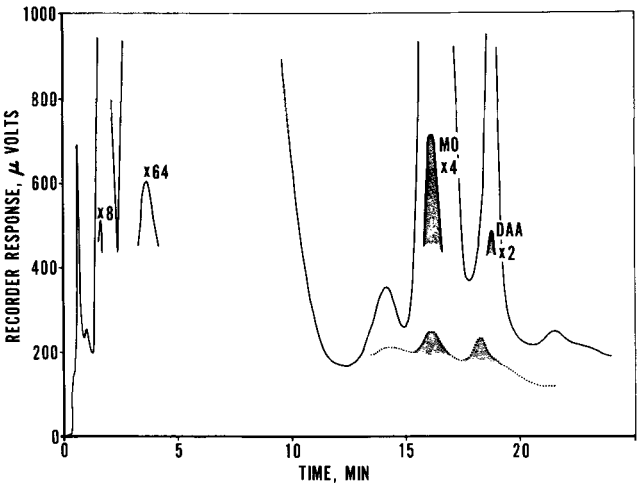


FIG. 4. Representative curve from the direct gas liquid chromatography method. MO = mesityl oxide and DAA = diacetone alcohol.

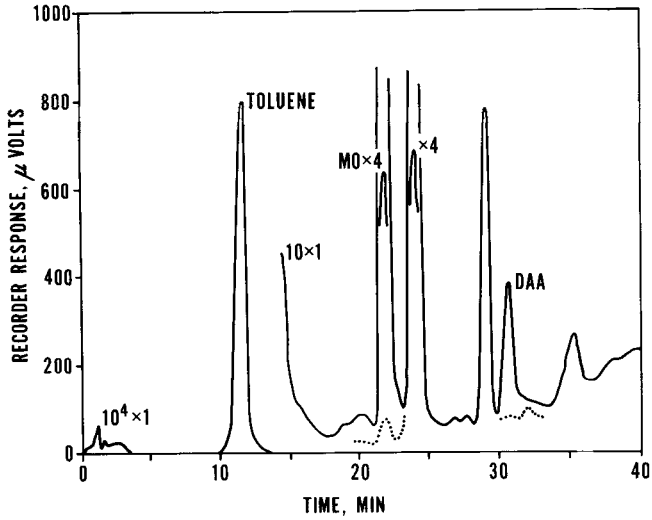


FIG. 5. Representative curve from the azeotropic distillation method. MO = mesityl oxide and DAA = diacetone alcohol.

TABLE I

Determination of Mesityl Oxide and Diacetone Alcohol in Acetone Extracted Cottonseed Meals and Flours by Direct Gas Liquid Chromatography (GLC) and Azeotropic Distillation Procedures

| Sample[a] number | Mesityl oxide, ppm | | Diacetone alcohol, ppm | |
|---|---|---|---|---|
| | Distillation | Direct GLC | Distillation | Direct GLC |
| 1 | 2 | 3 | ND[b] | ND |
| 2 | 4 | 4 | ND | ND |
| 3 | 300 | 220 | 60 | 70 |
| 4 | 15 | 20 | ND | 10 |
| 5 | 9 | 8 | 200 | 190 |
| 6 | ND | ND | ND | ND |
| 7 | 120 | 85 | 55 | 60 |

[a]Samples 1 and 6 were prepress cottonseed flakes which subsequently were extracted in our laboratories with acetone, hexane, and water (54:44:2) to remove residual oil and aflatoxins. Samples 2, 3, 4, and 5 were commercial, prepress, hexane extracted cottonseed meals which were extracted further in our laboratories with acetone and water (90:10) to remove aflatoxins. Sample 7 was a cottonseed flour commercially processed with acetone.

[b]ND = none detected.

made to volume with distilled water. A glass liner was prepared and installed in the gas chromatograph as described for direct GLC, with the exception that only a small plug of glass wool was placed in the liner. A 200 μliter aliquot of each of the standard solutions then was injected individually into the liner and analyzed according to Procedure A. Since the 200 μl aliquot of each standard solution contained the same amounts of mesityl oxide and diacetone alcohol as a 0.04 g sample of oilseed meal having 3, 10, 60, 200, and 300 ppm of these reagents, it was possible to construct the calibration curves shown in Figure 2 for converting peak area to ppm mesityl oxide and diacetone alcohol.

*Procedure B*: As described under "Standardization, Procedure A," standard solutions were prepared, in toluene, and contained 50, 250, 625, 1250, 2500, 5000, and 7500 mg mesityl oxide and diacetone alcohol/liter. Each standard solution (0.5 μliter) was analyzed as described under Procedure B. The amounts of mesityl oxide and diacetone alcohol contained in each 0.5 μliter aliquot of standard solution were the same as those which would be contained in a 0.5 μliter aliquot of the 1 ml toluene solution derived from the azeotropic distillation of 25 g oilseed meal or flour containing 2, 10, 25, 50, 100, 200, and 300 ppm of these reagents. Thus, a calibration chart was prepared as shown in Figure 3. The calibration lines were obtained by dividing peak areas for mesityl oxide and diacetone alcohol by peak areas for toluene and plotting these values relative to ppm.

## RESULTS AND DISCUSSION

To elute residual mesityl oxide and diacetone alcohol effectively from oilseed meals by direct GLC, it was necessary to provide an adequate and sustained flow of moisture to the sample. The injection of water directly into the glass wool in the liner was not satisfactory, since this released too much moisture over too short a period to elute the residual components effectively from the meal. Water-saturated molecular sieve, however, discharged moisture in a gradual manner, inducing a prolonged "steaming" effect which effectively released these components in the meal.

Figure 4 shows representative gas chromatographic curves obtained by direct GLC for samples of acetone extracted cottonseed meal. The solid curve (sample 3, Table I) indicates the presence of a large amount of mesityl oxide and a moderate amount of diacetone alcohol. The dotted curve (sample 2, Table I) indicates a small amount of mesityl oxide and no diacetone alcohol. It is important to note that the second peak of the dotted curve represents an unidentified material having a retention time slightly shorter than that of diacetone alcohol. Figure 5 shows the type of GLC curve obtained by analysis of the toluene solution derived from the azeotropic distillation of acetone extracted cottonseed meal (sample 3, Table I). Azeotropic distillation showed essentially the same order of magnitude of mesityl oxide and diacetone alcohol as the direct GLC method. Similarly, the dotted curve (sample 2, Table I) shows results which parallel those from direct GLC,

indicating a small amount of mesityl oxide and no diacetone alcohol.

Table I shows the results of analyses of seven samples of finely ground cottonseed meal by direct GLC and azeotropic distillation. Where concentrations of mesityl oxide were very high, direct GLC yielded values ca. 30% lower than those from azeotropic distillation. However, at lower levels, in the range of a few ppm, direct GLC appeared comparable to azeotropic distillation. Since the low levels of residual solvent are most meaningful in processing operations, use of rapid, direct GLC to monitor production would seem especially advantageous.

Agreement for diacetone alcohol was generally good with both methods of analysis. Values by direct GLC are somewhat higher than those obtained by azeotropic distillation, even though the required correction factors were applied to the results obtained by the distillation procedure. It is possible that more destruction of diacetone alcohol occurs during azeotropic distillation which requires higher operating temperatures and longer processing periods than direct GLC.

Thus, direct GLC provides a simple, rapid, and effective means of detecting residual mesityl oxide and diacetone alcohol in acetone processed oilseed meals and flours. For mesityl oxide determinations, the procedure is especially effective in the lower ranges of a few ppm. Diacetone alcohol determination appears to be quite reliable at both the high and low concentrations and does not require the use of a correction factor. Direct GLC should be especially useful to monitor production operations where many samples must be analyzed in minimum time.

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[Received October 25, 1974]