

TABLE II
COMPARISON OF ΔR_{Mr} (3-KETONE (5 α) \rightarrow 3-KETONE (5 β)) VALUES IN ADSORPTION
AND GAS-LIQUID PARTITION SYSTEMS

| Compound | ΔR_{Mr} (3-ketone (5 α) \rightarrow 3-ketone (5 β)) | | |
|------------------------------------|--|-----------------|-----------------------------|
| | Adsorption systems | | Gas-liquid partition system |
| | Alumina G | Silica Gel G*** | 6% QF-1 (250°) |
| Cholestan-3-one | - 0.26* | - 0.07 | - 0.04 |
| Pregnane-3,20-dione | + 0.03* | + 0.05 | - 0.04 |
| Androstane-3,17-dione | + 0.40** | + 0.14 | - 0.04 |
| 17 β -Hydroxyandrostan-3-one | + 0.12** | + 0.04 | - 0.04 |

* Eluant: chloroform-toluene (1:3).

** Eluant: chloroform-benzene (1:1).

*** Eluant: benzene-chloroform (1:3).

ketone for two adsorption systems and the gas-liquid partition system. It can be seen that in the partition system the 5 β -3-ketone is always eluted ahead of its 5 α -isomer with a consistent ΔR_{Mr} value of - 0.04. No such relationship is found in the adsorption systems.

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Some pitfalls in studies related to gas chromatography

This report discusses certain pitfalls in studies on natural products involving gas chromatography.

Qualitative and semiquantitative investigations on aroma-bearing constituents frequently involve procedures in which the constituents are ultimately obtained in an ether solution at very low concentrations, e.g., 1 l of solution containing 0.01% solute. The ether solution is then evaporated to a small volume (e.g., 1 ml or less)

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for subsequent gas chromatographic analysis. In contrast to strongly concentrated solutions^{1,2}, the percentage losses of solutes during evaporation of such weakly concentrated solutions may be quite large, as shown in Table I.

TABLE I
LOSSES OF METHYL ESTERS ON EVAPORATION OF SOLVENT FROM
ETHER SOLUTIONS BY VARIOUS METHODS

| Method | Method of solvent removal ^{a,b} | Over-all loss (%) of methyl esters of indicated <i>n</i> -acids | | | | |
|--------|--|---|----------------|----------------|----------------|----------------|
| | | C ₂ | C ₃ | C ₄ | C ₆ | C ₈ |
| 1 | SB → 30 ml → SBC → 1 ml | 95 | 85 | 96 | 78 | 43 |
| 2 | SB → 10 ml → SN → 1 ml | 96 | 91 | 97 | 76 | 36 |
| 3 | STC → 30 ml → SBC → 1 ml | 93 | 71 | 62 | 37 | 29 |
| 4 | SBC → 1 ml | 84 | 75 | 61 | 47 | 0 |

^a Each solution contained the following amounts dissolved in either 1 l (methods 1-3) or 30 ml (method 4) of ether: methyl propionate (b.p. 80°), 36 mg; methyl acetate (b.p. 57°), methyl *n*-butyrate (b.p. 103°), methyl caproate (b.p. 151°), and methyl caprylate (b.p. 192°), 20 mg each.

^b SB = steam bath. STC = 80 cm Stedman column (reflux ratio, 10:1). SBC = 25 cm micro spinning band column (reflux ratio, 7:1). SN = stream of nitrogen at or slightly below room temperature.

The solutes were a homologous series of esters present in very low concentrations in ether*; the concentrations were intended to simulate what may be encountered in a study of aromatic substances. The evaporation procedures included two methods sometimes encountered in the literature: simple boiling off on a steam bath and fractional distillation. The latter was conducted on two columns, one of high throughput and the other of low holdup. Since data on theoretical plate numbers were not available, the columns were conventionally operated under arbitrary conditions which represented a compromise between estimated efficiency and time of distillation. The over-all percentage loss for each procedure was determined by gas chromatography of the concentrates using a standard area-concentration curve for each component. Although minor quantitative discrepancies are observed in Table I, the magnitude of the losses can be approximated. Losses with all methods were high; considering time *vs.* efficiency, it is questionable whether the relatively slow (8 h) fractional distillation (method 3) would represent a significant advantage over the more rapid method 2 for routine use in the type of investigation under discussion. Except for methyl caprylate, the losses on the micro spinning column alone (method 4) were high considering that only 29 ml of solvent were removed. Although this may be indirect evidence that the larger column is more efficient, the exclusive use of this column is not feasible due to its relatively large holdup. Of course, lower losses might be obtained by using higher efficiency columns or reflux ratios or by some other procedure; however, the high ratios of solvent to solute make effective quantitative separation exceedingly difficult. In general, these data emphasize the need for caution in reporting semi-quantitative estimations or the absence of components in such studies without a knowledge of losses due to solvent removal.

A second pitfall concerns traces of impurities in solvents or reagents. Reports occasionally appear in which gas chromatograms from natural products are later

* This mixture would not be expected to form azeotropes³.

shown to have contained extraneous peaks from solvents or reagents⁴. The traces (*e.g.*, 0.005–0.03 %) of carbonyls, ethanol, etc., found in commercial anhydrous ether of the highest purity are concentrated to some extent on evaporation of the ether and may yield significant peaks on chromatograms. Distillation of such ether before use will reduce but not eliminate these peaks. Blank runs should be made to assure that the extraneous peaks are known or, if necessary, the ether may be further purified by an efficient chemical method, such as that of FIESER⁵.

Another pitfall is illustrated by a recent experience in this laboratory. In determining the phenols of tobacco smoke, a method was initially examined which yielded a low (about 10 %) recovery of added authentic phenol. Although a solvent (ether) evaporation step was included in the method, the over-all loss of phenol could not be accounted for entirely by the loss on solvent removal. Interest was centered on a preliminary step in the procedure in which an aqueous 0.5 % sodium hydroxide solution containing the phenol (17 mg per 200 ml solution) was continuously extracted for 24 hours with ether (750 ml) to remove extraneous, water- and ether-soluble, nonacidic substances. Gas chromatography of this ether extraction after concentration showed a significant amount (about 50 %) of the missing phenol therein. Further investigation showed that synthetic sodium phenolate gives a peak for free phenol on gas chromatographic analysis. The most plausible explanation was that continuous extraction with (wet) ether removed small but quantitatively significant amounts of sodium phenolate in a cumulative manner, thus contributing to the over-all loss.

Many other examples of pitfalls could be cited, *e.g.* the inadvertent removal of water-soluble components from ether solutions during washing to remove extraneous acid or base, and the failure to recognize extraneous peaks (usually from laboratory air) during headspace vapor analysis with flame ionization detectors. The continuing development of sensitive analytical instruments makes adequate recognition of methodological shortcomings increasingly necessary.

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