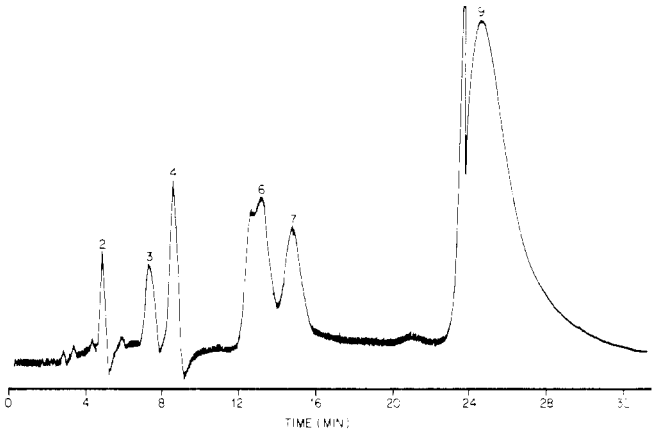

Figure 2. Gas chromatogram of extract from PCP by Jensen method before methylation. For peak identification, see Figure 1.

the Jensen and Rappe methods. The octa-CDE eluted with hexa-CDD, hepta-CDF eluted with hepta-CDD, and octa-CDF eluted with octa-CDD.

The technical PCP was analyzed by EC–GC before and after the methylation steps of Rappe and Jensen methods. If pre-CDD were present, conversion of the pre-CDD to the CDD during EC–GC would be expected; thus, the CDD level would be higher in the unmethylated sample than in the methylated sample. In previously reported results (Jensen and Renberg, 1972; Rappe and Nilsson, 1972) and in results found in this laboratory on other PCP preparations, the CDD levels were much higher before methylation than after methylation. In the technical PCP analyzed here, however, the octa-CDD and hepta-CDD levels were only slightly higher in the unmethylated sample, Figure 2, than in the methylated sample, Figure 1A. The levels were 1.4 and 1.2, respectively.

Two large new peaks appeared in the methylated sample (Figure 1A). By GC–MS analysis, these new peaks were identified as the methylated pre-CDD which convert to octa-CDD and hepta-CDD. The failure of most of the pre-CDD in the unmethylated sample to convert to the CDD suggests that this technical PCP may contain most of the pre-CDD in the iso pre-CDD form.

Jensen has suggested that the iso pre-CDD is an isomeric form of the pre-CDD in which the hydroxy group is in position 1 or 2 on the ring. Ring closure to the CDD is thereby prevented.

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Gas Chromatographic–Mass Spectrometric Studies of Ethoxyquin in Some Organic Solvents. I

Janneche Utne Skaare* and Hans K. Dahle

The stability of 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (ethoxyquin, EMQ) was studied in *n*-hexane and chloroform with emphasis on the color changes of the solutions and the quantitative changes during 1 month of storage in the absence of light. Visual observations, gas–liquid chromatography (GLC), and GLC combined with mass spectrometry (MS) were used as the methods of analysis. The antioxidant was found to be extremely labile on exposure to light, and in chloroform solutions an increase of color intensity was observed together with a 35–70% loss of GLC measurable EMQ, the tenfold dilute solutions (0.1 mg/ml) being the least stable. The ethoxyquin dissolved in *n*-hexane, however, was found to remain unchanged even after the storage period. In conclusion, *n*-hexane is therefore recommended as the solvent for use in analytical work and for extractions from biological systems containing ethoxyquin. GLC using a 3% SE-30 column operated at 160° has been found to be suitable for quantization of EMQ when residues are to be determined in food products for example. The mass spectra of the GLC peaks were examined for characteristic fragmentation patterns.

6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline, EMQ (ethoxyquin), is a remarkably effective antioxidant and is gaining increased application as a feed additive (Monsanto Chemical Co., 1961; Opstvedt et al., 1970, 1971). The necessity of understanding the chemistry of feed and food additives, and the fate of these substances in various biological systems, require that the compounds in question be studied under defined conditions, and that procedures for the exact determination of the additives and their breakdown products are available. In this paper we report some investigations on the choice of solvent for work with ethoxyquin

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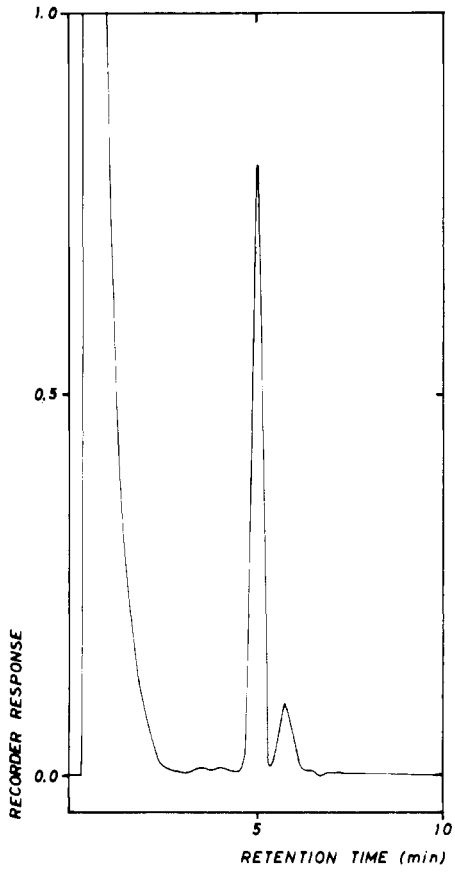


Figure 1. Gas chromatogram of 2 μl of a 0.1 mg/ml *n*-hexane solution of ethoxyquin analyzed on a 3% SE-30 column (160°) using a flame ionization detector.

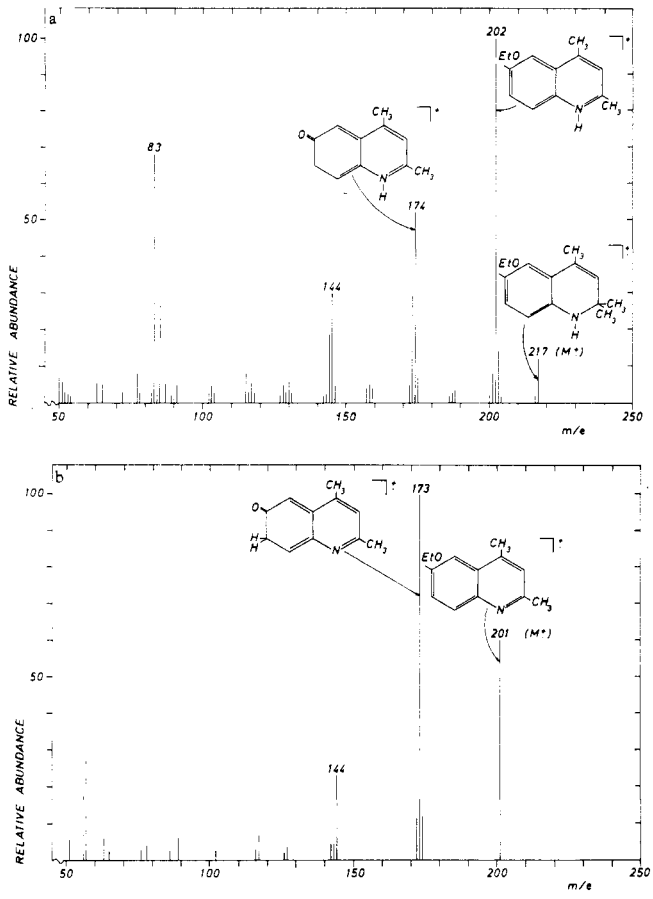


Figure 2. (a) Mass spectrum recorded at the GLC retention time of 5 min; (b) mass spectrum recorded at the GLC retention time of 5.8 min.

(EMQ) with special reference to the application of gas chromatography for the determination of the antioxidant. The identity of the GLC peaks has been confirmed by mass spectrometry and the mass spectra have been examined for characteristic fragmentation patterns.

MATERIALS AND METHODS

Reagents. Chloroform and *n*-hexane were of analytical grade and obtained from E. Merck, Darmstadt, Germany. Ethoxyquin (pure) was obtained from Koch-Light Laboratories Ltd. Standard solutions were made by transferring 100 mg to 100-ml volumetric flasks and diluting to volume with chloroform or *n*-hexane. Working solutions were made by diluting the standard solutions tenfold. All solutions were analyzed immediately following preparation and also after given time intervals during storage in darkness.

Instruments. *Gas Chromatograph–Mass Spectrometer.* An LKB Model 9000 mass spectrometer coupled to a gas chromatograph was used. The column was a 3 m × 3 mm i.d. glass tube packed with 3% SE-30 on acid-washed, DMCS-treated Chromosorb W, 80–100 mesh (Applied Science Lab., Inc., State College, Pa.). Operating conditions were as follows: temperatures, column at 185°, flash heater at 200°, molecular separator at 230°, and ion source at 270°; flow rates, helium (35 ml/min) was used as carrier gas. When recording gas chromatograms only, using the total-ion current detector (TICD) the ion source was operated at 20 eV. When scanning mass spectra the electron energy was changed to 70 eV automatically. For the quantitative work a Varian Model 2100 gas chromatograph equipped with a flame ionization detector was used. Operating conditions were as follows: temperatures, column at 160°, inlet at 180°, detector at 180°; flow rates (milliliters per minute), carrier gas (nitrogen) 30, hydrogen 30, air 300; range, 10^{-11}, 10^{-10}; attenuator setting, 8; 0.1–2.0 μg of the standard solutions was injected. EMQ (10 ng) injected on the column could be determined utilizing the described operating parameters.

RESULTS AND DISCUSSION

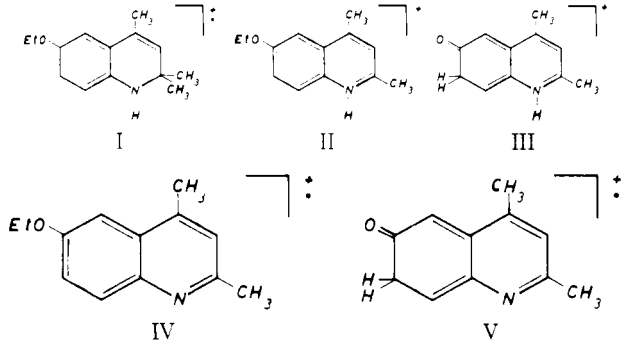
Characteristics of the Solutions. All of the ethoxyquin solutions were found to be sensitive toward light as they turned yellow-brown to dark-brown on exposure. Even when kept away from light the chloroform solutions were observed to change color, the degree of which depended on the concentration of ethoxyquin. The hexane solutions, however, remained about colorless even after 1 month of storage in darkness.

Concentration Changes during Storage. All the freshly prepared solutions had comparable concentrations as determined by GLC. Linearity was observed from 0.05 to 1 mg of EMQ/ml. The EMQ content of the hexane solutions as measured gas chromatographically remained unchanged during storage. In the chloroform solutions, however, decreasing concentrations were observed together with an increase in color intensity. For the standard chloroform solutions there was, after 1 month, an approximately 35% loss of GLC measurable EMQ, and for the dilute solutions a 70% loss was found. These observations were all in agreement with those reported by Contreras (1970).

GLC–MS. Figure 1 illustrates the gas chromatogram of the dilute solution of EMQ in hexane. The identity of the main GLC peak as 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (EMQ) was confirmed by mass spectrometry. The mass spectrum is shown in Figure 2a and the main characteristics of the spectrum can be summarized as follows: (1) the molecular weight is found to be 217, and the

structure of the molecular ion is illustrated in formula I. (2) The principal peaks below the parent ion region of the spectrum are at masses 202, 174, 144, and 83. The formation of some of the fragment ions can be explained as follows: the base peak of the spectrum is accounted for by the loss of a methyl radical in β position to the nitrogen atom of the molecular ion producing the very stable quinolinium structure (formula II) with a corresponding m/e value of 202. The intense peak at base peak minus 28 is attributed to the loss of a C_2H_4 neutral fragment resulting from a McLafferty rearrangement (McLafferty, 1959) and a possible structure of this fragment is illustrated in formula III. The fragment of mass 144 is a characteristic ionic feature of a quinoline derivative.

Doing repetitive scanning of mass spectra, the GLC peak at the retention time of 5.8 min is found to contain several quinoline derivatives. One of the most abundant components was identified by mass spectrometry to be 6-ethoxy-2,4-dimethylquinoline. The molecular weight of this component can be seen to be 201 from the mass spectrum in Figure 2b. The structure of the molecular ion is illustrated in formula IV. The relative abundance of the molecular ion is 60% as compared to 12% for ethoxyquin. These relative abundances reflect the different stabilities of the two molecular ions. The base peak of this spectrum is attributed to the molecular ion undergoing fragmentation via the McLafferty rearrangement. A suggested structure of the base peak of m/e 173 is illustrated in formula V.



I II III

IV V

When scanning mass spectra along the GLC profile of the chloroform solution of ethoxyquin, a mass spectrum with a molecular ion of mass 216 and a fragmentation pattern comparable to that of ethoxyquin was seen. The structural formula of this compound may well be that of a radical which has been shown to be present in the solution by electron spin resonance spectroscopy (ESR) (Skaare and Henriksen, 1975). A radical with the quinoline structure intact has also been proposed as the intermediate for the formation of the dimer of ethoxyquin which is thought to be the main oxidative product when EMQ is acting as an antioxidant (Gordon and Maddy, 1958). However, using the above-mentioned GLC conditions we did not succeed in isolating or identifying any dimer of EMQ.

The stability of ethoxyquin in the hexane solutions as compared to the observations of the color change and decrease in concentration when dissolved in chloroform indicates that hexane should be used as solvent in analytical and extraction work with ethoxyquin. Likewise, gas–liquid chromatography using an SE-30 column seems to be applicable as a method of analysis when the quantitative determination of EMQ is required. This method, applied to the quantitative estimation of ethoxyquin in various biological materials, will be published (Dahle and Skaare, 1975).

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Gas Chromatographic Determination of Ethoxyquin in Feed and Food Products. II

Hans K. Dahle and Janneche Utne Skaare*

A procedure is described for the estimation of the antioxidant ethoxyquin in feed and food products. It involves homogenization of the samples, extraction with methanol, and extraction of the antioxidant from the extract using n-hexane. Gas–liquid chromatography (GLC) with a flame ionization detector is used for quantitative determination, and GLC combined with mass spectrometry (MS) is applied for the confirmation of identity. Only the oxidation inhibitor 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline (EMQ), which has not been involved in the antioxidative processes, is determined gas chromatographically. Recovery studies adding ethoxyquin at levels of 100 ppm to fish meal, fish meat, and broiler meat showed that about 30% of the GLC measurable antioxidant was recovered, whereas approximately 70% was recovered from water.

Most of the methods described for the determination of ethoxyquin in feed and food products are based on fluorimetric principles built out for paper and thin-layer chromatography (Gordon and Maddy, 1958; Weilmann et al., 1972) or spectrophotometric equipment (Bickoff et al., 1956; Gordon et al., 1964; Witt et al., 1973). Color methods have also been used for routine work and some of these methods are reviewed in Fishing News International (1970). Gas–liquid chromatography (GLC) has previously not been reported as an analytical procedure for the determination of ethoxyquin. This could probably be due to the

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