A Capillary Gas–Liquid Chromatographic Method for Determination of Ethylenethiourea and Propylenethiourea in Hops, Beer, and Grapes

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A simple capillary gas chromatographic method was developed to assay propylenethiourea (PTU) and ethylenethiourea (ETU) residues in beer, hops, and grapes without derivatization. The limit of sensitivity was shown to be 0.005 ppm by using a flame photometric detector, FPD (sulfur mode, 394 nm). Residues of PTU/ETU in some commercial beers (1979) are also reported.

Ethylenethiourea (ETU) is a primary metabolite in environmental degradation (Engst and Schnaak, 1974; Marshall, 1977) and contaminant in the fungicides Maneb, Zineb, Nabam, and Amobam which are widely used for controlling plant disease. ETU is suspected to have caused various pathogenic effects like goiterogenic (Seifer and Ehrlich, 1948; Graham et al., 1973, 1975), carcinogenic (Innes et al., 1969), mutagenic (Seiler, 1974), and teratogenic (Khera, 1973; Lu and Staples, 1978).

In recent years concern has been expressed about its presence in foods and drinks. Various analytical methods have been reported for ETU determination. The analytical procedures involve extraction and cleanup on various columns (Otto et al., 1977), derivatization to 2-(butylthio)-2-imidazoline (Onley and Yip, 1971; Onley et al., 1977), 2-(benzylthio)-1-(pentafluorobenzoyl)-2-imidazoline (Nash, 1974), 2-(benzylthio)-1-(trifluoroacetyl-2-imidazoline (Newsome, 1972), 2-[*m*-[trifluoromethyl)-benzylthio]-1-(trifluoroacetyl)-2-imidazoline (king, 1977).

Newsome's method as reported in the literature (Nash, 1974) does not give consistent and quantitative recoveries. Using Nash's method for the determination of ETU in hops, beer, and grapes, we were faced with the problem of inconsistent and poor yields of *S*-benzyl-*N*-(pentafluorobenzoyl)-2-imidazolidinethione. Incomplete formation of derivatives were also observed by using King's and Onley's methods for the analysis of beer.

In this paper we report a simple cleanup method and determination of ETU and PTU in beer, hops, and grapes using a capillary GLC equipped with a flame photometric detector.

EXPERIMENTAL SECTION

Apparatus. A Carlo-Erba Fractovap Model 2101 AC equiped with FPD was used for GC analysis. The GC conditions were as follows: capillary quartz column coated with Carbowax M 20 (Hewlett-Packard); column length, 8 m; 0.25-mm i.d.; carrier gas, helium; flow rate, 4 mL/min; temperature program, 60–220 °C, 30 °C/min; detector temperature, 250 °C. This unit was connected with Hewlett-Packard Automation System 3385 A for data evaluation.

A Berthold liquid scintillation counter (BF betaszint 5000/300) with external standardization was used to measure radioactivity. Thin-layer chromatography plates were scanned for radioactive substances on a scanner supplied by Berthold-Frieseke GmgH, Karlsruhe, West Germany.

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Reagents. Ethylenethiourea-^{14}C (99% pure) was kindly supplied by the Bayer AG (West Germany). A scintillation liquid Hydroluma (I. T. Baker Chemia) was used for assaying ^{14}C. Ethylenethiourea (inactive) was purchased from Dr. Sand and I. Ehrenstorfer (Ausburg, West Germany). For TLC analysis ready-made silica gel plates (Merck; 0.25 mm thick) were used. Extrelut columns (Merck No. 11737) were used for cleaning up all extracts. The filling material of Extrelut columns is a wide porous Kieselgur with a corn-shaped structure and high porous volume. A column contains 14 g of the material, and a maximum of 20 mL of aqueous solution can be passed through the column. The principle of operation is a liquid–liquid extraction. The aqueous phase remains on the support, whereas lipophilic substances are extracted with organic solvents. In order to achieve better extractions of ETU/PTU from aqueous media, we added KF (salting out). The pH of extraction was adjusted with NH_4Cl, thus providing a faster elution with less organic solvent.

Procedure. ETU-^{14}C (0.018 μCi, 2.0 μg, 0.1 ppm) was applied to 20 mL of beer (prepared from hops untreated with any of the thiocarbamate fungicides). To this mixture, 0.6 g of NH_4Cl and 10 g of KF were added. After being stirred for 20 min with a magnetic stirrer, the solution was poured quantitatively on an Extrelut column. After 15 min the column was eluted with CH_2Cl_2 (analytical grade). The water phase remains on the support and ETU is eluted with 80 mL of CH_2Cl_2. The amount of ^{14}C in the eluate ranged from 85 to 95% in three successive experiments. The values obtained with PTU-^{14}C were also in the same range. The solvent was removed under reduced pressure, and the residue was taken in 0.1–0.5 mL of ethyl acetate (analytical grade) depending upon the concentration of ETU/PTU in sample for GLC analysis. Grob's splitless injection technique was used for quantitative determinations (insert purge delay 25 s). Grapes (25 g) (purchased from a local market) were treated with ETU-^{14}C (0.04 μCi, 4.3 μg, 0.17 ppm) and homogenized with an Ultraturrax (in an ice bath) in 160 mL of methanol–water (3:1) for 15 min. The homogenate was filtered through Whatman No. 1 paper in a Buchner funnel by using slight negative pressure. The filtrate was transfered to a 250-mL round-bottomed flask, and the solvent was evaporated to nearly 20 mL on a rotary evaporator. After the addition of 10 g of KF and 0.6 g of NH_4Cl, the concentrated solution was transferred to an Extrelut column and the method as described in the case of beer was followed. The recovery of ETU-^{14}C was 85–95%. A blank experiment showed practically no ETU or PTU was present in these grapes.

Air-dried hops (1 g) were treated with ETU-^{14}C (0.1 ppm) and homogenized with an Ultraturrax (in an ice bath) in 30 mL of methanol–water (3:1) for 15 min. The homogenate was filtered and concentrated to 1 mL in a

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Table I. ETU and PTU Residues in Various Commercial Beers (1979) from Different Breweries in West Germany (Extrelut Method)[a]

beer	ETU, ppm	PTU, ppm
Helles	0.097	0.037
Pils	0.26	0.12
Helles	0.12	0.06
Helles	0.16	n.d.[b]
Helles	0.12	n.d.
Helles	0.07	n.d.
Pils	0.05	0.01

[a] Detection limit: 0.01 ppm. [b] n.d. = not detectable.

Table II. Recoveries of ETU-^{14}C from Beer, Hops, and Grapes after Cleanup on the Extrelut Column (0.1 and 0.01 ppm)

sample	ETU, % (0.1 ppm)	ETU, % (0.01 ppm)
beer	90-95	85-92
hops	85-90	80-85
grapes	80-85	85-95

rotary evaporator. The residue was diluted to 20 mL with distilled water, and ETU was estimated in this solution as described above. The recovery was 85%.

Propylenethiourea (PTU) in various samples of beer was assayed by using the method as described for ETU determination. The estimations of ETU/PTU in various samples of commercial beers were performed in duplicate with the same procedure, and the average value is recorded in Table I.

RESULTS AND DISCUSSION

A variety of column packings and conditions have been used in the analysis of ethylenethiourea and its derivatives. Detectors include thermionic (Onley and Yip, 1971), flame photometric (Haines and Adler, 1973; Otto et al., 1977), and electron capture (Newsome, 1972; Nash, 1974; King, 1977). Quantitation of ETU as its halogenated derivative at low levels in various samples (Newsome, 1972; Nash, 1974; Onley at al., 1977) does not give consistent results. The yields of halogen derivative using Nash's method, for example (Scheme I), was less than 50% in the case of beer. The inhibition of quantitative derivatization may be attributed to the presence of various biological substances in beer. Although the yield of *S*-benzyl-*N*-(pentafluorobenzoyl)-2-imidazolidinethione (**3**) from *S*-benzyl-2-imidazolidinethione (**2**) can be improved by approximately 20% when pyridine is added to the solution of **2** prior to the addition of pentafluorobenzoyl chloride, the overall yield of **3** lies between 45 and 50% (calculated on the basis of ETU). Estimation of ETU in beer by GLC–FPD (Otto et al., 1977) gives a recovery of ETU of 65–70% (monitored with ETU-^{14}C). A capillary method for determining ETU without derivatization was also described (Hirvi et al., 1979). This method in its actual form was not applicable to the analysis of beer because poor cleanup was achieved. Too many coextractives are obtained after methanol homogenation and ethyl acetate extraction of freeze-dried beer. The recoveries of ETU added to various samples using the Extrelut column are given in Table II. The values are higher and the steps involved are less as compared to the reported method (Otto et al., 1977).

TLC examination of the eluates on silica gel plates in the solvent system [(a) benzene–MeOH (7:1); (b) $CHCl_3$–MeOH–NH_3 (85:14:1)] shows that no decomposition of ETU-^{14}C occurs during cleanup on Extrelut columns. Potassium fluoride and ammonium chloride both seem to play an important function in elution of ETU from the Extrelut column. Elution of ethylenethiourea from a beer which is not pretreated with KF and NH_4Cl through

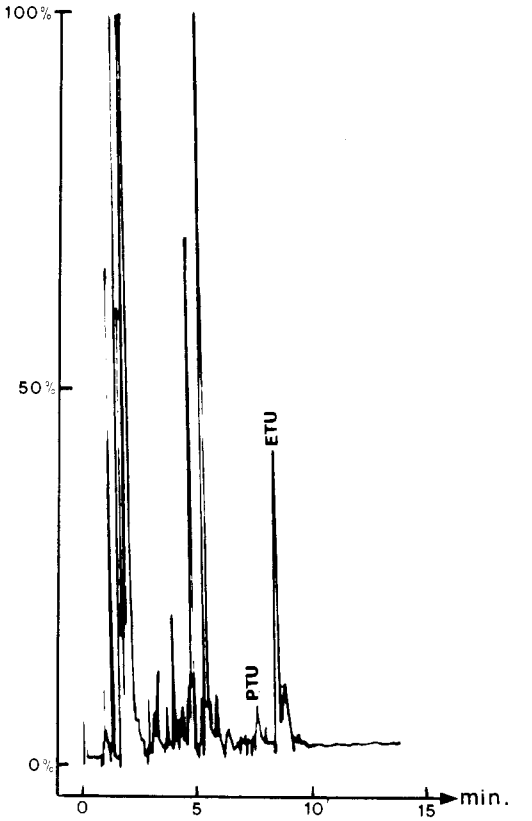


Figure 1. Gas chromatogram obtained from the extract of beer (after Extrelut cleanup) fortified with 0.1 ppm of ethylenethiourea.

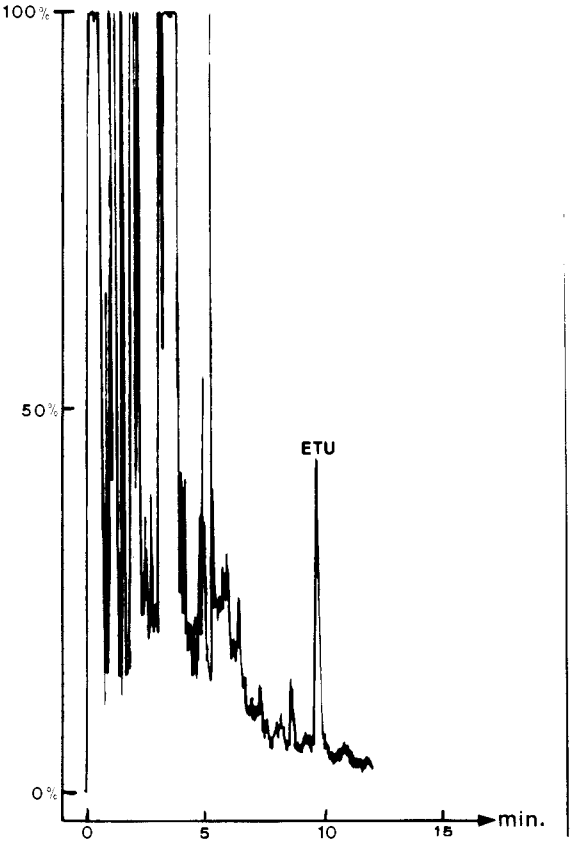
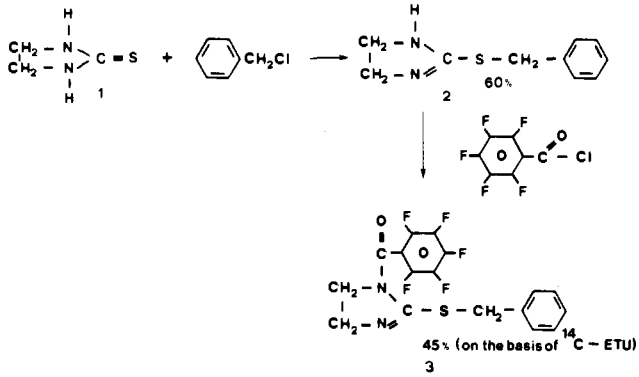


Figure 2. Chromatogram obtained from the extract of hops (after Extrelut cleanup) fortified with 0.1 ppm of ETU.

Scheme I. Reaction Scheme for the Derivatization of ETU with Benzyl Chloride and Pentafluorobenzoyl Chloride in Beer Extract



1 2 3

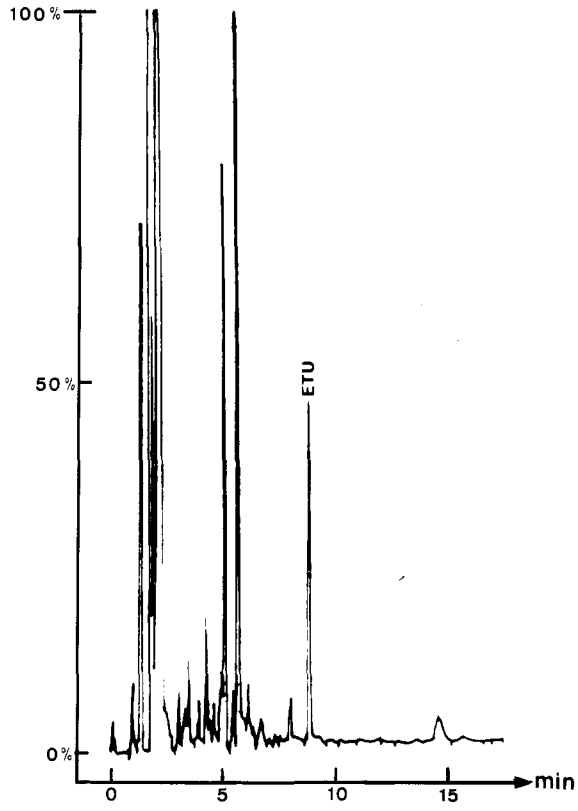


Figure 3. Gas chromatogram obtained from the extract of grapes (after Extrelut cleanup) fortified with 0.17 ppm of ETU.

an Extrelet column gives poor results (40%). It seems that both the salts help in setting ETU free from the biological matrix.

Figures 1,2, and 3 are typical gas chromatographs of beer, hops, and grapes extracts. The retention time of ETU in all the samples on an 8-m Carbowax 20M column (Hewlett-Packard) is 9.8–9.9 min. The retention time of PTU under the same conditions in 8.8–8.9 min. The area around ETU and PTU in gas chromatograms is free from interfering peaks. Considerable decrease of gas chromatographic sensitivity might be due to cracking of material inside the injector of GC. In this case replacement of insert is necessary. After cleanup, the samples must be quantitated as soon as possible because severe losses of ETU/PTU are observed if extracted samples are stored.

The confirmation of peaks at 9.8- and 8.9-min retention times as ETU and PTU was made by capillary GC–MS on a Finnigan Model 2010. Under optimal GLC conditions up to 1 ng of ETU or PTU can be detected (signal:noise ratio 2:1), so that the actual detection limit is 0.005 ppm. Figure 4 shows a gas chromatogram of 10 ng of ETU and PTU under the GC conditions described above.

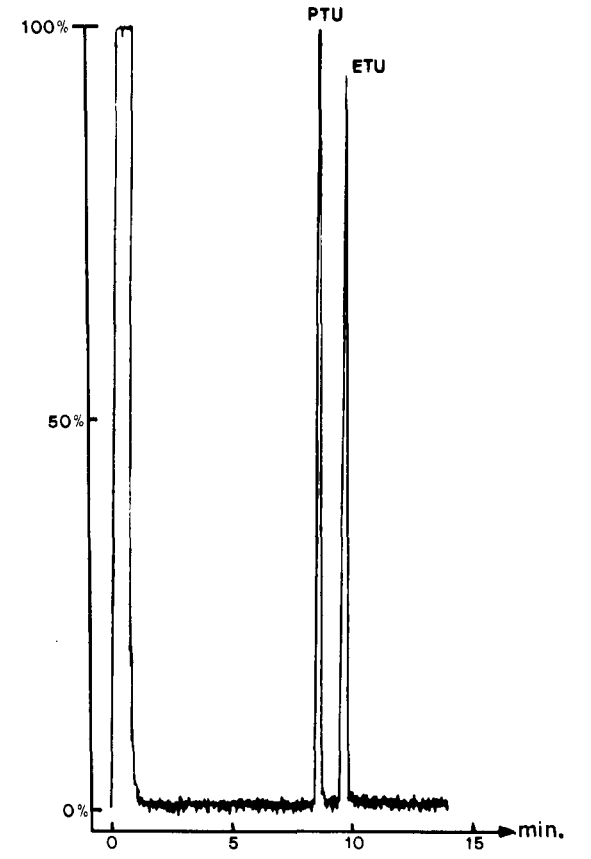


Figure 4. Gas chromatogram of the ETU and PTU mixture (10 ng each).

The amounts of ETU/PTU determined in several beer types manufactured in 1979 are summarized in Table I. In several cases the residues of ETU found were to be higher than the guide level of 0.1 ppm recommended by the FAO/WHO in Dec 1977. Research on the behavior of carbamates and ETU/PTU during beer manufacturing in our laboratory is in progress and will be published in a separate paper.

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Spectrophotometric Determination of Urea in Ammonium Nitrate Fertilizers

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A rapid spectrophotometric method is described for the determination of low concentrations of urea in ammonium nitrate fertilizer. Analyses require 30 min to process with numerous samples run sequentially. This method is based on the absorbance of the red color complex produced by thiosemicarbazide, diacetyl monooxime, and urea. The color is stable for at least 30 min, and the procedure follows Beer's law to 25 ppm (0.5 mg) when measured at 525 nm. Interferences have been noted with some amines which produce an orange color complex. Enhancement from ammonium ion is compensated for in the reference and standard. Due to this enhancement, the reference, standards, and samples must contain the same ammonium nitrate concentration.

Ammonium nitrate fertilizers sometimes are contaminated as a result of urea carry-over in ammonia/carbon dioxide off gas from urea synthesis to the neutralizer section of the ammonium nitrate process. Urea levels are normally very low in ammonium nitrate product, 1–20 ppm, but during process upsets may reach 200–400 ppm. Since urea contamination adversely affects ammonium nitrate quality, it is desirable to quickly and accurately determine the amount of urea present. Most colorimetric determinations for urea require lengthy reaction or color development times of 1 h or more and have low sensitivity. A time-saving and more sensitive method of determination would be of high practical importance, especially in quality control analysis.

Generally, spectrophotometric determination of urea falls into two main categories. The first category (indirect method) involves hydrolysis of urea to ammonia or an ammonium salt and carbon dioxide. The ammonia or ammonium ion then undergoes a color reaction. The most common hydrolysis procedure is catalysis with urease [AOAC Procedure 2.080 (Association of Official Analytical Chemists, 1980)].

The second category (direct method) involves the direct reaction of urea with another substance to produce a precipitate or color complex. One colorimetric method is based on the yellow-green color produced by *p*-(dimethylamino)benzaldehyde and urea in dilute hydrochloric acid solution. Interference from the ammonium ion is compensated for in the blank (Watt and Chrisp, 1954; Welcher, 1963). The concentration range of 50–240 ppm for this procedure fails to give satisfactory results with low urea concentrations as normally found in ammonium nitrate. The majority of work on the determination of low concentrations of urea (less than 50 ppm) has been done by clinical and diagnostic laboratories. Their work falls into four categories, depending on the amount of sensitizing agent, thiosemicarbazide, present. Fearon (1939) and Davidsohn-Wells (1963) discuss urea determination using diacetyl monooxime without thiosemicarbazide in which a yellow color complex is formed. Coulombe and Favreau (1963) and Crocker (1967) use thiosemicarbazide as a sensitizing agent with diacetyl monooxime to produce a red color complex. Natelson (1971) also discusses thiosemicarbazide as increasing sensitivity but decreasing specificity.

This new procedure is an adaptation of a clinical test for urea nitrogen in urine and blood, which reduces color development time. Sample preparation does not involve ammonia evolution as in the AOAC method. However, ammonium ion concentration is a factor in color intensity and must therefore be consistent in the reference, standards, and samples. The purpose of this work is to develop a spectrophotometric technique for the fast and sensitive determination of urea at low concentrations in ammonium nitrate fertilizers.

MATERIALS AND METHODS

Apparatus. *Spectrophotometer*: double beam with concentration mode, Hitachi Model 100-60 (NSA/Hitachi, Mountainview, CA 94043). *Constant temperature bath*: 75 ± 1 °C, Tempstir 66540 (Precision Scientific Co., Chicago, IL 60647) with an aluminum water bath (29.85 × 22.86 × 19.05 cm). *Test tubes*: 25 × 150 mm (Corning Glass Works, Corning NY 14830). *Wire basket*: round; 12.7-cm diameter × 15.24 cm.

Reagents. *Working Reagent.* This solution is prepared by thoroughly mixing equal volumes of color and acid reagents listed below. This solution is stable for 8 h. Urea Nitrogen Rapid Stat Color Reagent (8883-479552, Lancer, Foster City, CA 94404). Urea Nitrogen Rapid Stat Acid Reagent (8883-479548, Lancer, Foster City, CA 94404).

Urea Standards. 1.0 mg/mL urea standard: dissolve 1.0000 g of urea (ACS grade) to 1 L with distilled water. 0.10 mg/mL urea standard: dilute a 10-mL aliquot of 1.0 mg/mL standard to 100 mL.

Ammonium Nitrate. ACS grade.

Procedure. *Solid Samples.* Weigh 20.00 g of uncoated ammonium nitrate fertilizer sample in a 50-mL volumetric flask and dissolve with distilled water. Allow to warm to room temperature and then dilute to volume. If the sample is coated, then filter to obtain clear filtrate. Prepare a reference sample in the same manner by using reag-

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