AOAC Official Method 994.07
Ethyl Carbamate in Alcoholic Beverages and Soy Sauce
Gas Chromatography/Mass Selective Detection Method
First Action 1994 Final Action 1997

(Applicable to determination of ethyl carbamate levels of 50–350 μg/kg in distilled spirits, 40–160 μg/kg in fortified wines, 10–50 μg/kg in table wines, and 15–70 μg/kg in soy sauce.)

See Table 994.07 for the results of the interlaboratory study supporting the acceptance of the Method.

A. Principle

n-Propyl carbamate is added to test portion as internal standard, solution is diluted with H₂O, and then added to 50 mL solid-phase extraction column. Ethyl carbamate and n-propyl carbamate are eluted with methylene chloride. Eluate is concentrated in rotary evaporator. Concentrate is analyzed by gas chromatography/mass spectrometry in selected ion monitoring mode.

B. Apparatus

(a) Gas chromatograph/mass spectrometer (GC/MS).—With selected ion monitoring (SIM), autosampler, and Chemstation data handling system; or equivalent. Capillary column: 30 m × 0.25 mm id, 0.25 μm film DB-WAX fused silica (available from J & W Scientific, Folsom, CA 95630, USA) or 0.25 μm film Supelcowax 10 fused silica (available from Supelco, Inc., Bellefonte, PA 16823, USA).

Operating conditions: injector 180°C, helium carrier gas at 1 mL/min at 25°C, splitless injection. Temperature program: 40°C for 0.75 min, ramp at 10°C/min to 60°C, then 3°C/min to 150°C, post run, ramp to 220°C and then hold 4.25 min at 220°C. Typical retention time for ethyl carbamate is 23–27 min, and for n-propyl carbamate is 27–31 min. GC/MS interface: transfer line 220°C. MS parameters: manually tuned with perfluorotributylamine and optimized for lower mass sensitivity, SIM acquisition mode, solvent delay and group start time 22 min, dwell time/ion 100 ms.

(b) Rotary evaporator.—Buchi Rotovapor Model RE-120, or equivalent (i.e., Kuderna Danish). (Note: Recovery of ethyl carbamate from practice sample, C(g), must be 90–110% through entire procedure.)

(c) Flask.—Pear-shaped, 300 mL, single neck, with standard taper 24/40 joint at top.

(d) Concentrator tube.—4 mL, graduated, with standard taper 19/22 joint and standard taper 19/22 pennyhead stopper.

C. Reagents

(a) Acetone.—LC grade. (Note: Check each lot before use by GC/MS for absence of m/z 62, 74, and 89 responses.)

(b) Methylene chloride.—(Note: Check each lot before use by GC/MS at 200-fold concentration for absence of m/z 62, 74, and 89 responses.)

(c) Ethanol.—Anhydrous.

(d) Ethyl carbamate (EC) standard solutions.—(1) Stock solution.—1.00 mg/mL. Weigh 100 mg EC (≥99% purity) into 100 mL volumetric flask and dilute to mark with acetone. (2) Standard working solution.—10.0 μg/mL. Transfer 1 mL EC stock solution to 100 mL volumetric flask and dilute to mark with acetone.

(e) n-Propyl carbamate (nPC) standard solutions.—n-Propyl carbamate available from ICN Biomedicals, Irvine, CA 92713, USA. (1) Stock solution.—1.00 mg/mL. Weigh 100 mg nPC (reagent grade) into 100 mL volumetric flask and dilute to mark with acetone. (2) Standard working solution.—10.0 μg/mL. Transfer 1 mL nPC stock solution to 100 mL volumetric flask and dilute to mark with acetone. (3) nPC internal standard solution.—400 ng/mL. Transfer 4 mL nPC working standard solution to 100 mL volumetric flask and dilute to mark with H₂O.

(f) EC–nPC calibration standard solutions.—Dilute standard working solutions of EC, (d)(2), and nPC, (e)(2), with methylene chloride to obtain: (1) (100 ng EC and 400 ng nPC)/mL, (2) (200 ng EC and 400 ng nPC)/mL, (3) (400 ng EC and 400 ng nPC)/mL, (4) (800 ng EC and 400 ng nPC)/mL, and (5) (1600 ng EC + 400 ng nPC)/mL.

Table 994.07
Interlaboratory study results for determination of ethyl carbamate (EC) in alcoholic beverages and soy sauce by GC/MS method

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added EC, ng/g</th>
<th>Mean EC found, ng/g</th>
<th>Recovery of added EC, %</th>
<th>sᵣ</th>
<th>sᵣ</th>
<th>RSDᵣ, %</th>
<th>RSDᵣ, %</th>
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<tr>
<td>Distilled spirits</td>
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<td>2.25</td>
<td>4.78</td>
<td>4.47</td>
<td>9.49</td>
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<td></td>
<td>70</td>
<td>112</td>
<td>89</td>
<td>7.43</td>
<td>10.38</td>
<td>6.63</td>
<td>9.26</td>
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<td></td>
<td>320</td>
<td>329</td>
<td>87</td>
<td>13.24</td>
<td>28.03</td>
<td>4.03</td>
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<td>80</td>
<td>89</td>
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<td>7.00</td>
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<td>2.03</td>
<td>3.94</td>
<td>18.47</td>
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<tr>
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<td>3.29</td>
<td>9.70</td>
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</table>
(g) Practice test material.—100 ng EC/g in 40% ethanol. Transfer 1 mL EC standard working solution, (d)(2), into 200 mL volumetric flask and dilute with 40% ethanol until total weight is 100 g.

(h) Solid phase extraction column.—Disposable, prepacked with diatomaceous earth, 50 mL capacity, Chem Elut (available from Varian Sample Preparation Products, Sunnyvale, CA 94089, USA), or Extrelut QE Celite (available from EM Science, Gibbstown, NJ 08027, USA). (Note: Before analysis, check each batch of extraction columns for EC-nPC relative recovery and absence of m/z 62, 74, and 89 responses.) Prepare 100 ng EC/g test material, (g). Analyze 5.00 g practice test material as described in D(a), E, and F. Recovery of 90–110 ng EC/g is satisfactory. Varying degrees of absorbent fines may cause very slow flow rates affecting EC-nPC relative recovery. If after several trials 90–110% of practice test material value is not obtained, exchange column, or use recovery-corrected calibration curve to quantitate EC. To make corrected calibration curve, prepare standards as in (f) using 40% ethanol instead of methylene chloride. Analyze 1 mL calibration standard as described in D(a), E, and F, except do not add surrogate nPC solution. Construct new calibration curve, using EC/nPC ratios from extracted standards.

D. Preparation of Test Sample

Weigh into separate 100 mL beakers test materials using following amounts:

(a) Distilled spirits and fortified wines containing >14% alcohol (v/v).—5.00 ± 0.01 g.

(b) Table wines containing ≤14% alcohol (v/v).—20.00 ± 0.01 g.

(c) Soy sauce.—10.00 ± 0.01 g.

To each beaker add 1 mL nPC internal standard solution, C(e)(3), and H2O to obtain total weight 40.00 g.

E. Extraction

(Note: Perform extraction inside fume hood with adequate ventilation.)

Transfer diluted test portion from D to extraction column. Rinse beaker with 10 mL H2O and transfer rinsings to column. Let liquid absorb onto column 4 min. Elute 2× with 80 mL portions of methylene chloride. Collect eluate in 300 mL pear-shape flask. Evaporate eluate to 2–3 mL, using rotary evaporator in 30°C water bath. (Note: Do not allow extract to evaporate to dryness.) Transfer concentrate to 4 mL graduated concentrator tube, using 9 in. (228.6 mm) Pasteur pipet. Rinse flask with 1 mL methylene chloride and transfer rinsings to tube. Concentrate extract to 1 mL under gentle stream of nitrogen. Transfer concentrate to autosampler vial for GC/MS analysis.

F. GC/MS Analysis

(a) Calibration curve.—Inject 1 µL aliquots EC–nPC calibration standard solutions, C(f), into GC/MS. Plot EC–nPC area ratios for m/z 62 response on y-axis vs ng EC/mL on x-axis (i.e., 100, 200, 400, 800, 1600 ng/mL).

(b) EC quantitation.—Inject 1 µL concentrated extract from E into GC/MS system and calculate EC–nPC area ratio for m/z 62. Determine EC concentration (ng/mL) in extract, using internal standard calibration curve. Calculate EC concentration in test portion (ng/g [ppb]) by dividing amount of EC (ng/mL) in extract by weight of test portion (g).

(c) Confirmation of EC identity.—Determine if responses for m/z 62, 74, and 89 ions appear at EC retention time. These responses are diagnostic for principal fragments (M — C2H3)⁺ and (M — CH3)⁺, and molecular ion (M), respectively. EC presence is confirmed if relative ratios of these ions are within 20% of ratios for EC standard. Extract may need to be further concentrated to obtain sufficient response for m/z 89.

Reference: [J. AOAC Int. 77, 1530(1994)].

CAS-51-79-6 (ethyl carbamate)

Revised: March 1998