AOAC Official Method 997.12
Imidacloprid in Liquid and Solid Formulations
Reversed-Phase Liquid Chromatographic Method
First Action 1997
Final Action 2001

(Applicable to formulated products [technical, wettable powder, flowable, fertilizer, and granular formulations] containing imidacloprid as the only active ingredient.)

See Table **997.12** for the results of the interlaboratory study supporting the acceptance of the method.

Caution: *See Appendix B*, safety notes on safe handling of organic solvents and safe handling of test material. Imidacloprid is inhalation, ingestion, and absorption hazard; use protective gloves.

A. Principle

Test portion is dissolved in methanol or acetonitrile (depending on type of formulation). Imidacloprid is determined by liquid chromatography. Peak areas of analyte and standard are compared using propiophenone as an internal standard.

B. Apparatus

(**a**) *Liquid chromatograph*.—Generating >7 MPa (>1000 psi), with a spectrophotometer capable of measuring absorbance, *A*, at 252 nm, and peak area integrator. Operating conditions: column temperature, ambient; flow ca 1.2 mL/min (ca 1800 psi); recorder speed 0.5 cm/min; recorder range 10 mV; injection volume ca 1 μL for fertilizers and ca 10 μL for all other formulations; *A* range, 1.0 AUFS. Retention times: imidacloprid, ca 2.1 min; propiophenone (internal standard), ca 4.1 min. Pump the LC mobile phase through the column for >15 min, until system is equilibrated (flat baseline).

(**b**) *Chromatographic column*.—250 × 4.6 mm id packed with 5 μm octyldecylsilane-bonded silica gel.

(**c**) *Filters*.—0.45 μm porosity, solvent compatible.

(**d**) *Mechanical shaker*.

(**e**) *Sampler*.—Riffle type.

(**f**) *Ultrasonic bath*.

C. Reagents

(**a**) *Acetonitrile*.—LC grade or distilled in glass.

(**b**) *Water*.—LC grade or distilled in glass.

(**c**) *Methanol*.—LC grade or distilled in glass.

(**d**) *LC mobile phase*.—Acetonitrile–H_2O (60 + 40, v/v) thoroughly degassed.

(**e**) *Internal standard solution*.—2.5% (w/v) propiophenone (free of interferences at imidacloprid retention time) in methanol.

(**f**) *Imidacloprid reference standard*.—(Available from Bayer Corp., Agriculture Division, PO Box 4913, Hawthorne Rd, Kansas City, MO 64120-0013, USA.) Store refrigerated when not in use.

D. Preparation of Imidacloprid Standard Solutions

(*Note*: Imidacloprid standard solution is prepared differently depending on formulation being analyzed.)

(**a**) *For technical wettable powder and flowable formulations*.—Prepare imidacloprid standard solution S_1 as follows: Accurately weigh to the nearest 0.0001 g ca 0.2 g imidacloprid reference standard, **C**(**f**), into ca 150 mL bottle. Add 5.0 mL internal standard solution, **C**(**e**), and 95 ± 10 mL acetonitrile. Cap bottle with polyethylene-lined lid, sonicate contents in ultrasonic bath 1 min, and mix thoroughly by inverting bottle rapidly. From this solution, transfer 100 ± 20 μL into 2 mL auto-injection vial, add ca 1–1.5 mL acetonitrile, cap, and mix thoroughly.

(**b**) *For fertilizer formulations*.—Prepare imidacloprid standard solution S_2 as follows: Accurately weigh to the nearest 0.0001 g ca 0.2 g imidacloprid reference standard, **C**(**f**), into ca 250 mL bottle. Add 5.0 mL internal standard solution, **C**(**e**), and 95 ± 10 mL methanol, **C**(**c**). Cap bottle with a polyethylene-lined lid, sonicate contents in ultrasonic bath 1 min, and then mix thoroughly. From this solution transfer 100 ± 20 μL into 2 mL auto-injection vial, add 1.0–1.5 mL acetonitrile, cap, and mix thoroughly.

(**c**) *For granular formulations*.—Prepare imidacloprid standard solution S_3 as follows: Accurately weigh to the nearest 0.0001 g ca 0.2 g imidacloprid reference standard, **C**(**f**), into a 250 mL bottle. Add 5.0 mL of internal standard solution, **C**(**e**), and 150 ± 10 mL acetonitrile, **C**(**a**). Cap bottle with a polyethylene-lined lid, sonicate contents in ultrasonic bath 1 min, and then mix thoroughly. From this solution, transfer 100 ± 20 μL into 2 mL auto-injection vial, add ca 1–1.5 mL acetonitrile, cap, and mix thoroughly.

E. Preparation of Test Solutions

(**a**) *Technical, wettable powder, and flowable materials*.—Accurately weigh to the nearest 0.0001 g test portion containing ca 0.2 g technical material, ca 0.3 g of 75% wettable formulation, or ca 1.0 g flowable formulation into ca 150 mL bottle. (*Note*: Technical material and 75% wettable powder formulation do not require mixing or grinding.)

Thoroughly mix flowable formulation using spatula, spoon or similar device. Ensure that any "caked" material is dislodged from

Table 997.12 Interlaboratory study results for determination of imidacloprid in pesticide formulations by liquid chromatography

Statistic	Technical	75% WP	20% Flowable	Fertilizer	Granules
Mean	97.73	74.96	21.44	0.51	1.05
s_r	0.49	0.34	0.09	0.01	0.03
r	1.38	0.95	0.26	0.02	0.08
s_R	1.12	1.25	0.48	0.03	0.03
R	3.14	3.49	1.35	0.08	0.10
RSD_r, %	0.50	0.45	0.43	1.50	2.85
RSD_R, %	1.15	1.66	2.25	5.71	3.28
No. of laboratories	12	12	10	11	12

the bottom or sides of the container and is re-dispersed. Vigorously shake material 1 min before weighing. Add 10 mL H_2O only into bottle containing flowable formulation and swirl contents to mix. Sonicate 1 min in ultrasonic bath.

Do not add H_2O to technical, wettable powder, fertilizer, and granular formulations.

Pipet 5.0 mL internal standard solution, **C**(**e**), into bottle. Add 95 ± 10 mL acetonitrile, cap bottle with a polyethylene-lined lid, sonicate in ultrasonic bath 1 min, and mix thoroughly.

Filter each solution through separate filters, **B**(**c**). Transfer 100 ± 20 μL each filtrate into separate 2 mL auto-injection vials. Add ca 1–1.5 mL acetonitrile, cap, and mix thoroughly. Proceed to **F**, *LC Determination*. Use imidacloprid standard solution S_1, **D**(**a**), for analysis.

(**b**) *Fertilizer*.—Pour entire laboratory sample across center of riffle type sampler, collecting riffled portions in metal trays. Select one of the portions to continue riffling until riffled portion is 35–45 g. Weigh the entire portion to nearest 0.01 g into ca 250 mL bottle. Ensure that all dust in laboratory sample bottle is transferred to 250 mL bottle.

Pipet 5.0 mL of internal standard solution, **C**(**e**), into bottle. Add 95 ± 10 mL methanol and cap bottle with a polyethylene-lined lid. Shake bottle horizontally 60 min on mechanical shaker. Let solution settle after shaking. Filter portion of each solution through separate filters, **B**(**c**), into separate 2 mL auto-injection vials. Proceed to **F**, *LC Determination.* Use imidacloprid standard solution S_2, **D**(**b**), for analysis.

(**c**) *Granular formulations*.—Pour entire laboratory sample across center of riffle type sampler, collecting riffled portions in metal trays. Select one of the portions to continue riffling until riffled portion is 35–45 g. Weigh the entire portion to nearest 0.01 g into 250 mL bottle. Ensure that all dust in laboratory sample bottle is transferred to 250 mL bottle.

Pipet 5.0 mL of internal standard solution, **C**(**e**), into bottle. Add 150 ± 10 mL acetonitrile and cap bottle with a polyethylene-lined lid. Shake bottle horizontally 60 min on a mechanical shaker. Let solution settle after shaking. Filter each solution through separate 0.45 μm filters, collecting 100 ± 20 μL in separate 2 mL auto-injection vials. Add ca 1–1.5 mL acetonitrile, cap and mix thoroughly. Proceed to **F**, *LC Determination*. Use imidacloprid standard solution S_3, **D**(**c**), for analysis.

F. LC Determination

Adjust operating parameters so that elution times of imidacloprid and propiophenone peaks are within 1.9–2.5 min and 4.0–4.4 min, respectively.

Make repetitive injections of the appropriate standard solution from **D** and calculate the response ratios by dividing imidacloprid peak areas by that of the internal standard peak area. Response ratios for the standard injections (R) must agree within ±1% (±0.5% of their average) for 2 consecutive injections before proceeding with analysis.

Inject each test solution in duplicate (no more than 3 tests , i.e., 6 injections) between bracketing standard injections. Calculate response ratio of analyte (M) by dividing internal standard peak areas by imidacloprid peak areas. Response ratios of test solution injections must agree within ±1.0%. Otherwise, repeat determination starting with injections of imidacloprid standard solution.

Re-inject imidacloprid standard solution. Average response ratios of standard injections (R_{av}) immediately preceding and following test solution injections. These must agree within ±1.0% or repeat any portion of determination which does not meet this criterion.

G. Calculation

Calculate imidacloprid concentration (%) in test portion as follows:

$$\text{Imidacloprid, \%} = (W_s/W) \times \textit{purity} \times (PI_t/PS_t) \times (PS_{std}/PI_{std})$$

where W_S = weight of imidacloprid reference standard, g; W = weight of test portion, g; *purity* = percentage purity of imidacloprid reference standard; PI_t = peak area of imidacloprid from test portion; PS_t = peak area of internal standard from test portion; PS_{std} = peak area of internal standard from standard solution; and PI_{std} = peak area of imidacloprid from standard solution.

Reference: *J. AOAC Int.* **81**, 344(1998).

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