Determination of Formetanate Hydrochloride in Strawberries

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A method was established for determining formetanate hydrochloride in strawberries by reversed-phase high-performance liquid chromatography (HPLC). Formetanate hydrochloride was extracted from strawberries with acidified organic solvents (acetonitrile:HCl:ethyl acetate), and the extract was purified with a solid-phase extraction (SPE) column followed by a strong cation-exchange (SCX) cartridge, and analyzed by HPLC with UV diode array detection at λm of 254 nm. The mobile phase was 0.01 M, pH 8 ammonium phosphate buffer:acetonitrile (70:30). Formetanate was quantitated by the internal standard method. The percent recovery averaged 87.4 ± 2.2 (0.167 mg kg^{-1}), 89.0 ± 2.1 (1.67 mg kg^{-1}), and 91.9 ± 3.2 (16.7 mg kg^{-1}), the limit of determination (LD) was 0.018 mg kg^{-1}, and the limit of quantitation (LQ) was 0.18 mg kg^{-1}.

Keywords: *Formetanate hydrochloride; strawberries; residue; high-performance liquid chromatography (HPLC)*

INTRODUCTION

Formetanate hydrochloride [3-(dimethylamino)methyleneaminophenyl methylcarbamate hydrochloride] is an acaricide/miticide. It acts by inhibition of acetylcholinesterase and is effective for the control of spider mites, rust mites, certain aphids, thrips, lygus bugs, leaf hoppers, slugs, and snails on horticultural, agronomic, and ornamental plants. Recently, the trend in overall usage of the pesticide has been increasing. However, food monitoring data are lacking because the methodology available is time consuming; for example, gas chromatographic methods (U.S. Food and Drug Administration, 1968; Jenny, 1974) are based on the hydrolysis of formetanate hydrochloride to 3-aminophenol, which is then converted to a brominated derivative for measurement with an electron-capture detector. One high-performance liquid chromatography (HPLC) method (Lawrence, 1981), which depends on multiple liquid−liquid partitionings and pH cycling from alkaline to acid and back to alkaline for cleanup, consumed many solvents and much time. Another HPLC method (Niemann, 1993) was developed with coupled-column cation-exchange liquid chromatography. In our method, most of the pigments were eliminated by extraction with a solution of ethyl acetate added to acidified acetonitrile. Then, a solid-phase extraction (SPE) column followed by a strong cation-exchange (SCX) cartridge were used for cleanup, and HPLC analysis was done with the commonly used 100 RP-8 (5 μm) column. It is very practical to analyze several different compounds successively without changing the HPLC column. The mobile phase was 0.01 M $NH_4H_2PO_4$, pH 8 (range between 7.6 and 8.4). The solvent used by Niemann (1993; acetonitrile:HCl) extracted more pigments than did our solvent (acetonitrile:HCl:ethyl acetate). Furthermore, formetanate was quantitated by the method of internal standard, which was added just before HPLC analysis.

MATERIALS AND METHODS

Chemicals. Formetanate hydrochloride (99.3%) was a gift from Schering (Wolfenbûttel, Germany), and chlorotoluron {99.4%, 3-(3-chloro-*p*-tolyl)-1,1-dimethylurea} was purchased from Cluzeau (St. Foy La Grande, France). The SPE column (nonpolar C18 octadecyl column, 6 mL/500 mg, catalog no. Al-121020-52) and SCX cartridge (aromatic sulfonic acid, strong cation-exchange cartridge, 6 mL/500 mg, catalog no. 6803-2607) were purchased from Varian (Harbor City, CA) and from Whatman (Maidstone, England), respectively. All other chemicals used were of analytical reagent grade.

Preparation of Standard Solution (SM1: 100.0 μg mL^{-1}). First, 10.00 mg of formatanate hydrochloride was weighed into a 10-mL beaker and dissolved with the aid of 0.5-mL additions of water as needed. The formetanate hydrochloride was then transferred by acetonitrile rinses to a 100-mL volumetric flask and diluted to volume in acetonitrile.

Preparation of Internal Standard Solution (SM2: 100.0 μg mL^{-1}). Ten milligrams of chlorotoluron was weighed into a 10-mL beaker, transferred by acetonitrile rinses to a 100-mL volumetric flask, and diluted to volume in acetonitrile.

The two standard solutions remain stable for 1 month when stored at 4 °C in the dark.

Preparation of Basic Buffer (0.01 M $NH_4H_2PO_4$, pH 8). First, 0.580 g of $NH_4H_2PO_4$ was weighed into a 500-mL volumetric flask, dissolved with ultrapure water, and diluted to volume. Then, an additional 5.0 mL of NaOH (1 M) was added. After filtration through a 150-mL fritted glass filter and a nylon filter (47 mm, 0.2 μm, Whatman), the pH of the filtrate (7.6−8.4) was verified with a pH meter (digital pH meter, model 691 Roucaire, Ω Metrohm, with probe and combination glass electrode) that was calibrated by a 2-point standardization in pH 4 and 7 standard buffer solutions.

Preparation of Other Solutions. The other solutions used were: acetonitrile:water (97.5:2.5, v/v; M1), acetonitrile: 1.2 M HCl (1000:1.5, v/v; M2), ethyl acetate:M2 (40:60, v/v; M3), ethyl acetate:M1 (95:5, v/v; M4), and M1:1.2 M HCl (1000: 1.5, v/v; M5).

Extraction and Cleanup Procedures. All glassware was rinsed with 10 mL of solution M3. Thirty grams of strawberries were blended twice, with 100 and 80 mL of solution M3, with a low-speed blender (Waring, with 1 L glass jar) for 1 min and filtered through a 150-mL fritted glass filter (porosity,

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Scheme 1. Established Analytical Procedure for Formetanate Hydrochloride in Strawberries

Sample (30 g)
↓ blend with M3 (100 mL and 80 mL)
filtrate
dilute to 250 mL in M3
Extract (50 mL)
↓ evaporate to about 5 mL
SPE Column
↓ activate with 2 × 5 mL methanol & 3 × 5 mL M3
elute with M2 (4 × 5 mL)
Eluent
↓ evaporate to about 1 mL
SCX Cartridge
↓ activate with 2 × 5 mL methanol & 3 × 5 mL M4
wash with M4 (3 × 3 mL)
elute with M5 (4 × 5 mL)
Eluent
↓ evaporate to dryness
dissolve in M1 (1.80 mL) & SM2 (0.20 mL)
HPLC

4) under reduced pressure. For the recovery study, 5, 50, and 500 μg of formetanate hydrochloride were pipetted into the strawberries and covered with aluminum foil (acetone rinsed; dull side down). After 1 h at ambient temperature, the spiked samples were mixed and filtered. All the filtrate was collected in a 250-mL volumetric flask and adjusted to volume with solution M3. Fifty milliliters of extract was placed in a 100-mL round-bottom flask and concentrated under reduced pressure at 30 °C to ~5 mL with a rotavapor (Büchi 111). The concentrated 5-mL extract was applied onto an SPE column that was activated with methanol (2 × 5 mL) followed by solution M3 (3 × 5 mL). The pressure was adjusted to give a flow rate of ~3 mL min^{-1}. The column was then eluted with solution M2 (4 × 5 mL). This fraction was collected in a small round-bottom flask (50 mL) and concentrated under reduced pressure at 30 °C to ~1 mL. Then, 1 mL of the concentrated extract was applied on an SCX cartridge that was activated with methanol (2 × 5 mL) followed by solution M4 (3 × 5 mL) at ~3 mL min^{-1}. The round-bottom flask was rinsed with two additional 0.25-mL aliquots of fresh solution M2. The cartridge was washed with solution M4 (3 × 3 mL) at ~3 mL min^{-1}. If the solution M4 flowing out from the cartridge was not colored, it was discarded before elution with solution M5 (4 × 5 mL) into a small round-bottom flask (50 mL); if not, to improve recovery rate, it was necessary to collect solution M4 and evaporate it. The residue was dissolved with solution M5. This solution was then used for elution as previously indicated.

The phase M5 was concentrated under reduced pressure at 30 °C to dryness. Then, 1.80 mL of solution M1 and 200 μL of internal standard solution (SM2) were added to the round-bottom flask, and the flask was covered with aluminum foil (acetone rinsed, allowed to dry, dull side down), sealed with parafilm, and shaken gently for 30 s with ultrasound. Then, the determination of formetanate hydrochloride was carried out by HPLC (Scheme 1).

High-Performance Liquid Chromatography (HPLC). A high-performance liquid chromatograph equipped with a UV detector (Waters 991 Photodiode Array Detector; at λm, 254 nm), injector (Waters U6k, with a 25-μL injection loop), pump system (Waters 600 Multisolvent Delivery System), and integrator {NEC Powermate SX Plus (programme:PDA Waters)} was used. The separation was performed on a Lichrospher 100 RP-8 column (5 μm, 250 × 4.6 mm; E. Merck, Darmstadt, Germany) with basic buffer:acetonitrile (70:30) as the mobile phase at a flow rate of 1.0 mL min^{-1} at room temperature.

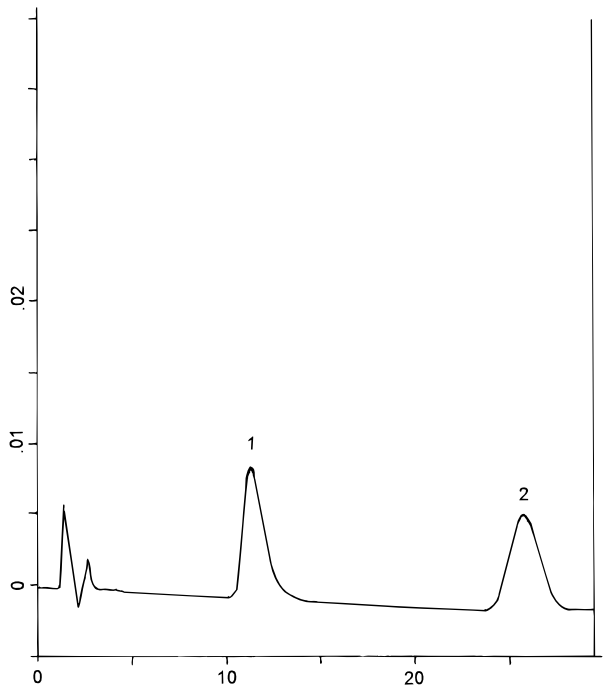

Figure 1. Chromatogram of formetanate hydrochloride standard. Separation conditions are described under Materials and Methods. Peaks: 1, formetanate (10.00 μg mL^{-1}, t_R = 11.34 min); 2, chlorotoluron (10.00 μg mL^{-1}, t_R = 25.82 min).

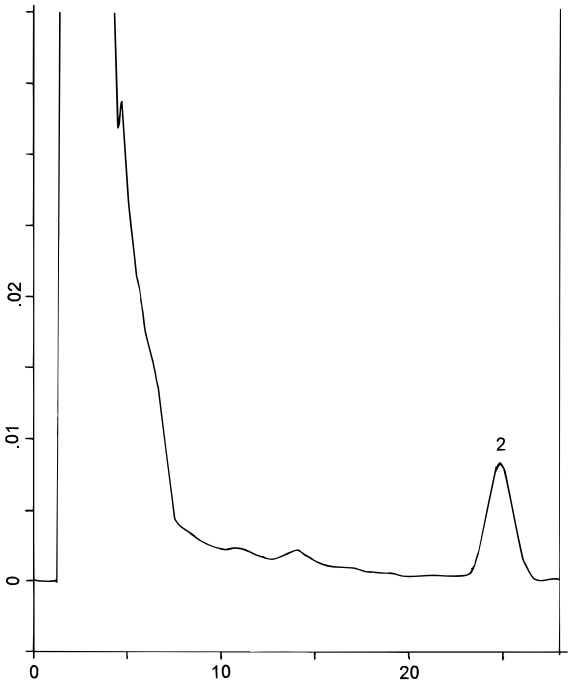

Figure 2. Chromatogram of blank control of formetanate hydrochloride in strawberry. Peak 2, chlorotoluron (10.00 μg mL^{-1}, t_R = 25.82). There was no formetanate in blank control.

RESULTS AND DISCUSSION

The chromatograms are shown in Figure 1 (standard), Figure 2 (blank control), and Figure 3 (positive control fortified at 5.00-mg kg^{-1} level).

Internal Standard Calibration Curve. To prepare standard solutions of 0.5, 1, 5, 10, 25, and 50 μg mL^{-1}, 0.50-, 1.00-, 2.50-, and 5.00-mL aliquots of stock solution (SM1), respectively, were diluted in separate 10-mL

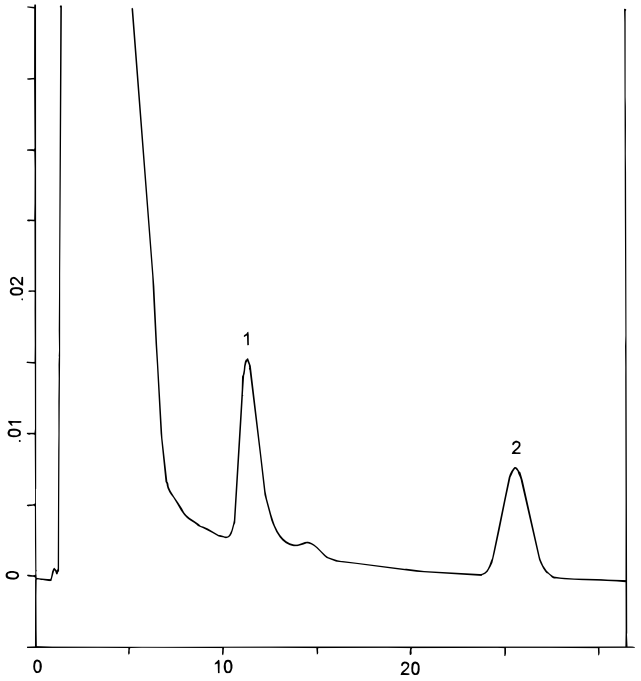

Figure 3. Chromatogram of positive control fortified with formetanate hydrochloride (5.00 mg kg^{-1}). Peaks: 1, formetanate (t_R = 11.34 min); 2, chlorotoluron (10.00 μg mL^{-1}, t_R = 25.82 min).

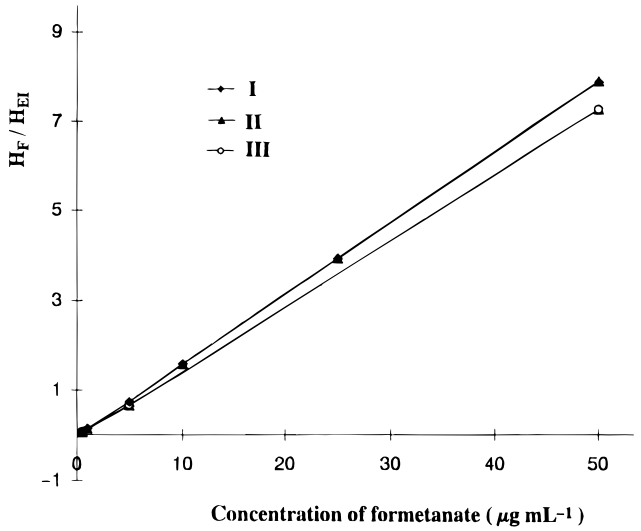

Figure 4. Calibration curves based on standard solutions and spiked strawberry extract. I, standard I; II, standard II; III, spiked strawberry extract.

volumetric flasks in which 1.00 mL of internal standard solution (SM2) was included; each flask was diluted to volume with M1. Also, 0.50- and 1.00-mL aliquots of the 10.0-μg mL^{-1} solution were diluted in separate 10-mL volumetric flasks in which 1.00 mL of internal standard solution (SM2) was included; again, each flask was diluted to volume in M1. A representative curve, shown in Figure 4 (I), has the regression equation $y = 0.1582x - 0.019$ ($r = 0.99998$).

Recovery Study. Three levels of recovery (five times each level in 3 days) were determined, and the results are shown in Table 1. The average regression equation was $y = 0.43661x - 0.03915$ [$r = 0.99995$; y is the ratio of peak height between formetanate and chlorotoluron, H_F/H_{EI}; x is the concentration (mg kg^{-1}) of formetanate hydrochloride added to the samples] (Figure 4). At the 95% confidence level, the F-test statistic was consistent with the assumption of no difference in variance between the lower and higher recovery data sets (calculated $F = 0.36 < 6.39$ critical F for 4; 4 degrees of freedom).

Table 1. Recovery of Formetanate Hydrochloride in Strawberries (Percent)

	concn		
sample	0.167 mg/kg^{-1}	1.67 mg/kg^{-1}	16.7 mg/kg^{-1}
1	86.1	87.3	95.5
2	85.4	85.8	93.0
3	88.0	90.2	94.6
4	91.0	85.6	89.9
5	86.6	95.8	86.6
av (n = 5)	87.4	89.0	91.9
SD	2.2	2.1	3.2
CV (%)	2.5	2.4	3.5

Table 2. Repeatability of Formetanate Hydrochloride Determination

	ratio H_F/H_{EI}					
concn (mg kg^{-1})	first day	second day	third day	av (five spiked samples)	SD	CV (%)
0.167	0.06390	0.06333	0.06747	0.06485	0.0016	2.5
		0.06532	0.06422			
1.67	0.64353	0.66483	0.70660	0.65580	0.031	4.8
	0.63268	0.63134				
16.7	7.53540	7.46599	7.09626	7.25493	0.29	4.0
	7.34031		6.83668			

Table 3. Limit of Determination and Limit of Quantitation

av r	av a	av b	av $-b/a$	SDB	LD (mg kg^{-1})	LQ (mg kg^{-1})
0.99995	0.43661	−0.03915	0.08771	0.018	0.056	0.18

Repeatability. Each level of recovery test was repeated twice at the same time and three times on different days. The ratio H_F/H_{EI} was calculated, and the results are shown in Table 2. Actually, it was better to use the peak height for the response instead of peak area because there was a contaminant peak close to the tail end of peak 1 (formetanate).

Limit of Determination (LD) and Limit of Quantitation (LQ). The set of five calibrations from the recovery study showed that the regression equation was $y = 0.43661x - 0.03915$ ($y = ax + b$). Accordingly, calibration data and statistics were used to compute the LD and LQ in terms of the standard deviation of calibration blanks (SDB):LD = 3 × SDB, and LQ = 10 × SDB. The calibration blank (mg kg^{-1}) at zero response was calculated from $-b/a$, and the results are shown in Table 3.

Stability Study. Four samples of 10.00 mg of the standard of formetanate hydrochloride (99.3%) were weighed into separate 100-mL volumetric flasks, dissolved, in either acetonitrile:water (99:1), distilled water, acidified buffer (0.4 M $NH_4H_2PO_4$, pH 3), or basic buffer (0.4 M $NH_4H_2PO_4$, pH 8), and conserved at 4 °C. Each day, 1 mL of the four different stock solutions were pipetted into separate 10-mL volumetric flasks in which 1.00 mL of internal standard solution (SM2) was included. Each flask was diluted to volume with each solvent and analyzed by HPLC each day, except for the basic buffer that was analyzed by HPLC continuously. The results presented in Figures 5 and 6 show that formetanate hydrochloride was stable in acetonitrile:water (99:1, v/v). On the other hand, it was hydrolyzed in the 0.4 M basic buffer, and the regression coefficient of the curve was near to 1. Its regression equation was: ln (area F/area C) = 0.3276 − 0.0070 t (min), r = 0.9981.

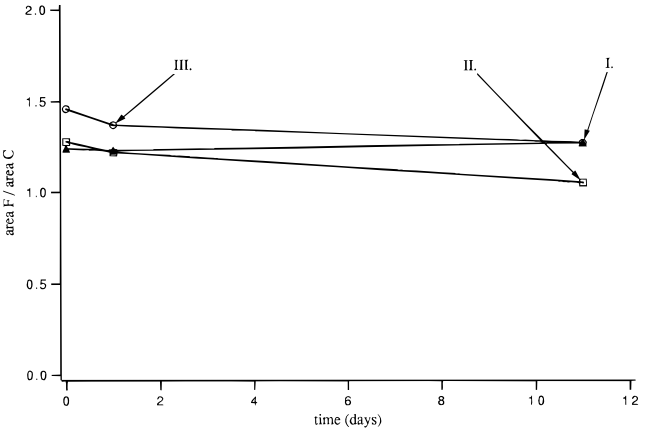

Figure 5. Stability of formetanate: I, in acetonitrile:water (99:1, v/v); II, in distilled water; III, in acidified buffer (0.4 M $NH_4H_2PO_4$, pH 3).

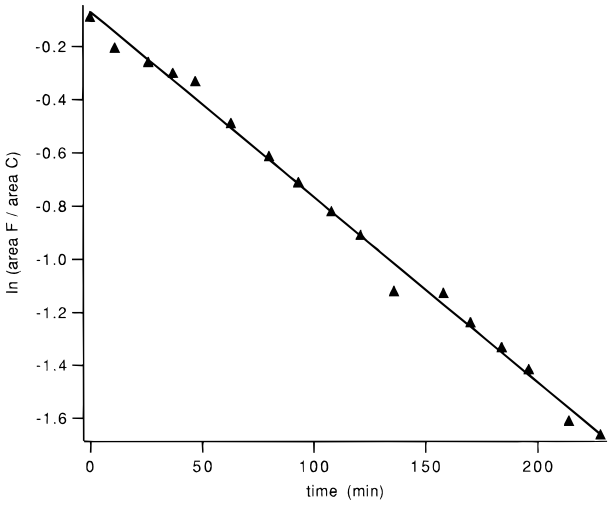

Figure 6. Stability of formetanate in basic buffer (0.4 M $NH_4H_2PO_4$, pH 8).

To verify the integrity of formetanate in the mobile phase 0.01 M $NH_4H_2PO_4$ (pH 8), a set of standard solutions of formetanate hydrochloride (0.5, 1, 5, 10, 25, and 50 μg mL^{-1}) were prepared as was done to determine the internal standard calibration curve, which was determined each day. The results (shown in Table 4 and Figure 4) show that formetanate was stable and therefore, there is no problem in analyzing it under these conditions.

Selection of Solvent of Extraction. When only acidified acetonitrile was used for extraction, formetanate could not be well separated from pigments. Nevertheless, the detection of formetanate after HPLC separation was not a problem; recovery of added formetanate was ~50% when samples were fortified at 0.30−5.00 mg kg^{-1}. So, formetanate hydrochloride could be trapped by some colored coextractives, and stability was verified. To prevent the column and detector from being overloaded and improve the recovery, a set of proportions between ethyl acetate and M2 were used for the extraction. Finally, solution M3 was chosen.

Table 4. Response to Formetanate in Standard Solution of Alkaline Mobile Phase

concn (μg mL^{-1})	H_F/H_{EI} first day	H_F/H_{EI} second day
0.5	0.07419	0.07359
1	0.14384	0.14354
5	0.73726	0.73716
10	1.58396	1.58510
25	3.93050	3.92851
50	7.89299	7.87647
regression eq	$y = 0.1582x - 0.019$ (curve: standard I)	$y = 0.1579x - 0.017$ (curve: standard II)
r	0.99998	0.99991

The method developed here was applied in our laboratory to analyze 22 unknown samples that have field-incurred for residues. Residue was found in three samples. The samples came from the experimentations in strawberries with Dicarzol 200 (2.5 kg ha^{-1}) and were collected at different times after spraying, during the summers of 1992 and 1993, by the French National Office of Crop Protection.

In summary, the method described was practical, precise, and easy to use with reversed-phase HPLC for determination of parent residues in strawberries.

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