Journal of Chromatography, 643 (1993) 249–260
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2780

Review

Determination of benomyl and its degradation products by chromatographic methods in water, wettable powder formulations, and crops

Raj P. Singh

Chemistry Department, Brock University, St. Catharines, Ontario L2S 3A1 (Canada)

Mikio Chiba*

Research Station, Agriculture Canada, Vineland Station, Ontario L0R 2E0 (Canada)

ABSTRACT

Chromatographic methods, used for the determination of methyl [1-(butylcarbamoyl)-1H-benzimidazol-2-yl]carbamate (benomyl) and methyl 1H-benzimidazol-2-ylcarbamate (carbendazim or MBC) in water, wettable powder (WP) formulations, and crops have been discussed. Because of the instability of benomyl in water and common organic solvents, most methods reported for the analytical determination of benomyl use an indirect approach. Since the kinetics of degradation of benomyl in water and common organic solvents is important in the development of analytical methods of benomyl, kinetic rates of various degradation reactions of benomyl are also discussed. The methods, based on the conversion of benomyl into MBC, and stabilization of benomyl in the presence of excess butyl isocyanate (BIC), will over-estimate benomyl with wide range of errors. Since MBC is a natural degradation product of benomyl and is present in different media at varying concentrations with benomyl, it should be determined individually with the intact concentrations of benomyl.

CONTENTS

* Corresponding author.

1. INTRODUCTION

Methyl [1-(butylcarbamoyl)-1H-benzimidazol-2-yl]carbamate (benomyl) is one of the most widely used systemic fungicides. For many years, benomyl has been successfully used for the control of many plant diseases [1–6]. Recently, the National Research Council (U.S.A.) has estimated the use of benomyl at two million lbs (1 lb $\approx$ 0.45 kg) of active ingredient per year [7]. Benomyl is a non-volatile white crystalline solid which has small solubility in water [8].

Because of its extensive use and suspected carcinogenic activity, determination of benomyl (and its degradation products) in environmental water, soil extracts, and crops is required. Accurate determination of benomyl (and MBC) in wettable powder formulations and pathological solutions (used in the studies to determine the efficacy and mode of action of benomyl) is also required. Instability of benomyl in common organic solvents and water has been used as an excuse by many researchers to determine benomyl as its degradation product MBC (after quantitative conversion of benomyl to MBC) in crops and other matrices. Early methods of benomyl determination are reviewed by Slade [9] and Gorbach [10]. In this review we critically discuss different chromatographic methods that are reported in the literature for the determination of benomyl and its degradation products. In most methods reported for benomyl determination, benomyl is converted into a stable compound, normally into a degradation compound of benomyl. Therefore, kinetics of degradation of benomyl in water (at different pH values) and common organic solvents is important in the development of analytical methods for the determination of benomyl. Keeping this in mind, in this paper, we have also reported kinetics of the degradation of benomyl.

2. KINETICS OF BENOMYL DEGRADATION

2.1. Degradation in partially aqueous and aqueous solutions

In aqueous solutions benomyl is not stable and converts to methyl 1H-benzimidazol-2-ylcarbamate (carbendazim, more commonly MBC), 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole (STB) and, 1-(2-benzimidazolyl)-3-*n*-butylurea (BBU), depending upon the pH of the solutions. In highly alkaline solutions (pH > 13) MBC slowly converts to 2-aminobenzimidazole (2-AB). The degradation of benomyl in aqueous solutions can be represented as depicted in Fig. 1.

The distribution profiles of various degradation products of benomyl, in benomyl saturated aqueous solutions of different pH values, have been depicted in Figs. 2 and 3.

The kinetics of decomposition of benomyl to its different degradation compounds was first studied by Calmon and Sayag [11,12], in methanol–water (50:50, v/v) using a spectrophotometric method. On the basis of the absorbance *vs.* time plots, these authors were able to determine the pseudo-first-order rate constants of benomyl decomposition to MBC, STB, and BBU, graphically. However, due to the low solubility of benomyl in water, Calmon and Sayag had to use a mixture of aqueous buffers and methanol to dissolve large amounts of benomyl in the working solution, which was required to study its decomposition kinetics by the spectrophotometric method. On the basis of a detailed investigation of the UV spectra of solutions at the end of reaction, they concluded that in the pH range 2.5–7.0, the final product was MBC. Their plots of the logarithms of observed pseudo-first-order rate constants (k_{obsd}) against pH indicated that in strongly acidic media (pH < 2.5) the decomposition reaction was inhibited by hydronium ions whereas over the pH range 2.5–7.0 the reaction rate was pH independent.

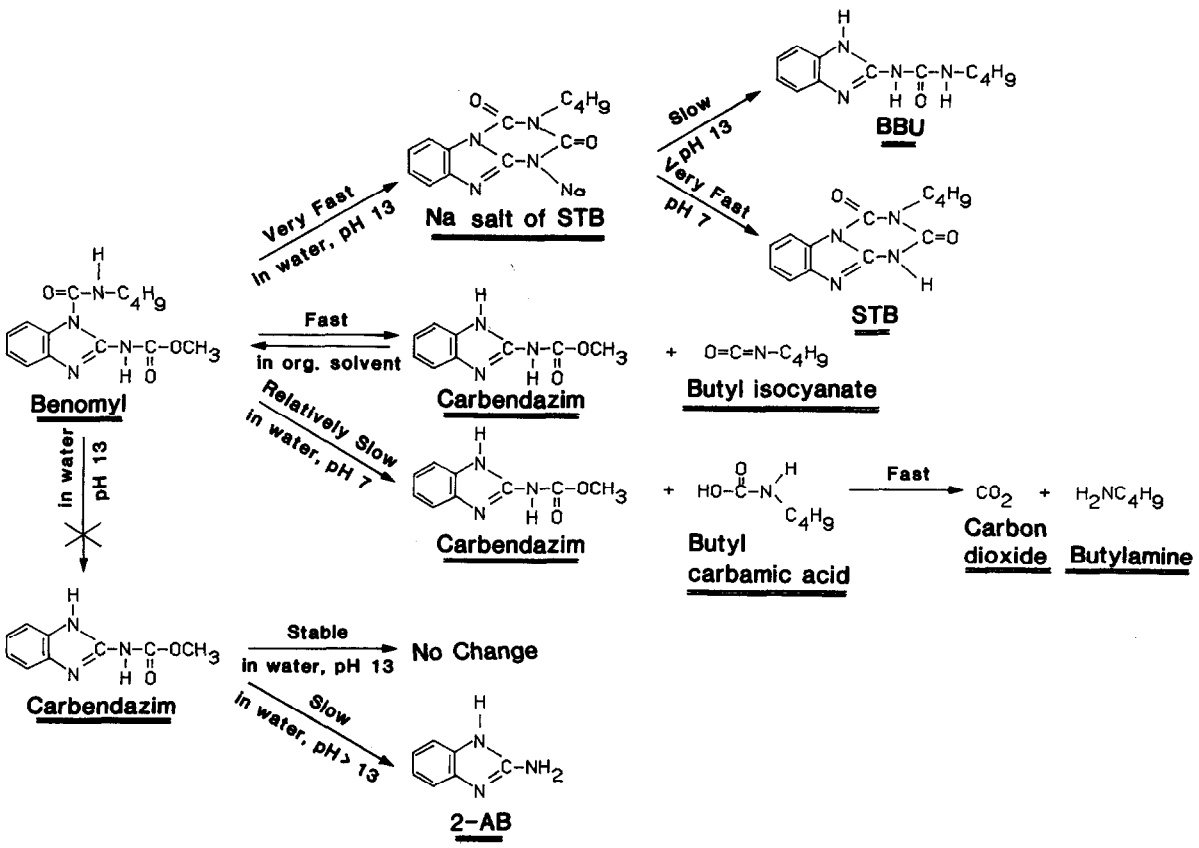

Fig. 1. Degradation of benomyl in aqueous and organic solvents at room temperature.

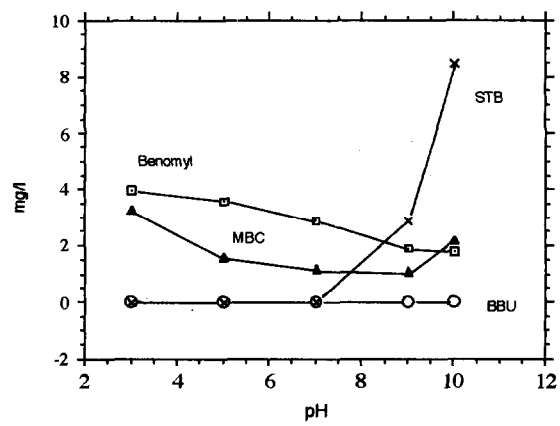

Fig. 2. Composition of benomyl (wettable powder) saturated aqueous buffers of pH 3–10, with respect to benomyl, MBC, STB and BBU.

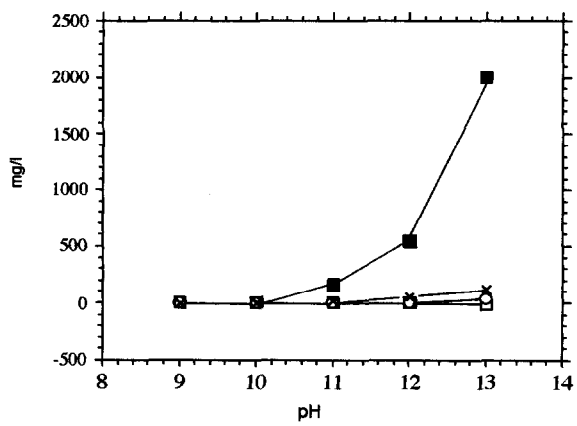

Fig. 3. Composition of benomyl (wettable powder) saturated aqueous buffers of pH 9–13, with respect to benomyl (□), MBC (×), STB (■) and BBU (○).

The conversion of benomyl to STB and BBU in alkaline medium was first studied by White *et al.* [13]. These authors reported that in alkaline medium benomyl was converted into STB, which after its formation, converted slowly to BBU in standing solutions. The amount of BBU in solution increases with time, temperature, and alkalinity. The work of White *et al.* [13] may have prompted Calmon and Sayag [12] to study the kinetics and mechanism of benomyl degradation to STB and BBU in alkaline media. Their studies were carried out in aqueous alkaline buffers–methanol (50:50, v/v), at constant ionic strength maintained at 1.0 mol/l. On the basis of their investigation Calmon and Sayag [12] concluded that the kinetics of hydrolysis of benomyl in alkaline media proceeded through several reaction mechanisms. In mildly alkaline media (pH < 12), conversion of benomyl to STB proceeds via $E_{1C}B$ elimination mechanism, followed by a fast cyclization. In strongly alkaline media (pH > 12), benomyl is converted into STB via a dianion. The conversion of STB to BBU occurred in very strongly alkaline media (pH > 13.5) and followed a first order rate kinetics with respect to hydroxide ion.

A knowledge of stability of benomyl in water at different pH values is important not only to agricultural and analytical chemists but also to plant pathologists. Although the work of Calmon and Sayag [11,12] on the kinetics of degradation of benomyl at various pH values in aqueous buffers–methanol (50:50, v/v) media have shown a trend of benomyl degradation, their results cannot be taken quantitatively for the development of analytical methods of benomyl determination.

Keeping this in mind Singh *et al.* [14] studied the kinetics of decomposition of benomyl in pure aqueous solutions of different pH values, using reversed-phase high-performance liquid chromatography (RP-HPLC). In the RP-HPLC method, benomyl in aqueous solutions was directly analyzed using a suitable mobile phase. The decrease in benomyl peak height with time was used to calculate the kinetic rate constants (k) according to the equation

$$k = 2.303/\Delta t \log C_1/C_2$$

where C_1 is the benomyl peak height at time t_1, C_2 is the benomyl peak height at time t_2, and $\Delta t = t_2 - t_1$.

The rate constant (k) of benomyl conversion to

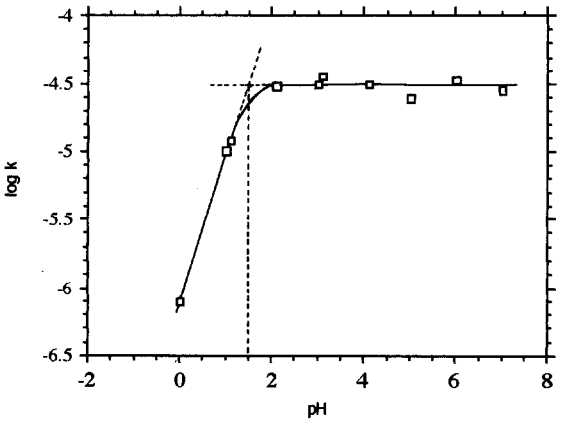

Fig. 4. Plot of log k_{obs} (rate constant of benomyl decomposition to MBC) *versus* pH of aqueous solutions.

MBC in the aqueous solutions remained nearly constant between pH 2 and 7 with mean k at $(3.16 \pm 0.38) \cdot 10^{-5}$ s^{-1}. However, in acidic solutions, below pH 2, protonation of benomyl (at the nitrogen of benzimidazole group) starts taking place. The plot of logarithm of k *versus* pH (pH 0–7) is shown in Fig. 4. In pure aqueous solutions the trend in the log k *vs.* pH plot was similar to that observed by Calmon and Sayag [11]. However, the mean k at $(3.16 \pm 0.38) \cdot 10^{-5}$ s^{-1}, in pure aqueous media in th pH range 2–7, was 60% lower than $5.0 \cdot 10^{-5}$ s^{-1}, the mean value of k reported by Calmon and Sayag [11], in aqueous buffers–methanol (50:50, v/v) in the same pH range.

A pK_a value of 1.5 was graphically determined for ionization of benomyl-H^+, in pure aqueous solutions on the basis of the plot in Fig. 4.

Kinetic study of degradation of benomyl in pure auqeous alkali solutions (pH ⩾ 13) was also carried out by us [15]. In a solution of sodium hydroxide (0.125 mol/l) of pH 13, a very fast conversion of benomyl to STB was observed. The first order rate constant (k_{obs}) for this reaction was estimated at approximately 0.01 s^{-1}. This value of k_{obs} is about 9 times higher than that reported by Calmon and Sayag [12] in methanol–sodium hydroxide (50:50, v/v) solution of pH 13. The value of k_{obs} for the degradation of STB to BBU depends upon the alkalinity and temperature. Again, the degradation of STB to BBU was 4 times higher in pure aqueous

solution than in methanol–sodium hydroxide (50:50, v/v) solution of the same pH.

Degradation of MBC to 2-AB was observed only in strong alkali solutions. In 1.0 mol/l sodium hydroxide, the value of k_{obs} was $1.32 \cdot 10^{-4}$ s^{-1} at 22°C. However, at high temperature, this reaction was faster. A value of approximately 0.034 s^{-1} was obtained in 0.125 mol/l sodium hydroxide at 80°C.

2.2. Degradation in common organic solvents

Rapid degradation of benomyl to MBC in common organic solvents was first reported by Chiba and Doornbos [16]. Calmon and Sayag [17] confirmed their finding and determined the rate constants of the decomposition of benomyl to MBC. They further reported that the conversion of benomyl to MBC proceeds by spontaneous intramolecular catalysis. The values of observed rate constants do not show any correlation with the existing empirical solvent parameters. However, the observed rate constant data can be explained in terms of solvent–solute interactions. The spontaneous intramolecular catalysis is markedly slowed down by the presence of water.

Chiba and Cherniak [18] have studied the kinetics of the decomposition of benomyl in common organic solvents such as chloroform, dichloromethane, ethyl acetate, benzene, ethanol, methanol, and dioxane using a spectrophotometric method at 25°C. These authors discovered that the reaction of the decomposition of benomyl was reversible (Fig. 1), *i.e.*,

$$\text{Benomyl}\ (k_{21}) \xleftrightarrow{\text{organic solvents}} \text{MBC} + \text{BIC}\ (k_{12}).$$

where k_{12} and k_{21}, are the specific rates of benomyl degradation and reformation, respectively [18], and BIC is butyl isocyanate.

The values of the specific rate constants k_{21} and k_{12} were determined for benomyl decomposition reaction in different (above-mentioned) organic solvents. The values of k_{12} showed no correlation with existing solvent parameters. However, k_{21} was found to be larger for less polar solvents. The reversible nature of the decomposition reaction was different in different solvents. The largest percentage of intact benomyl at equilibrium was found for benzene while the smallest intact concentration of intact benomyl was found in methanol.

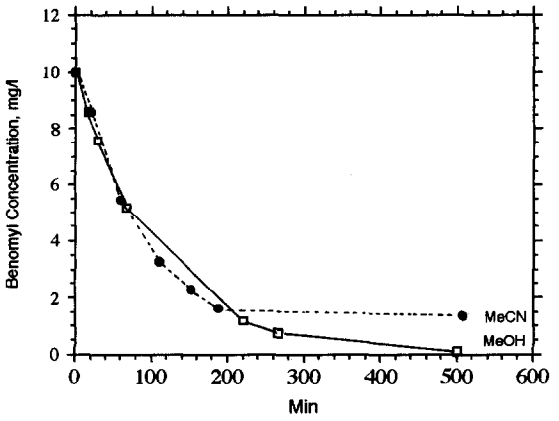

Fig. 5. Decomposition kinetics of benomyl to MBC in acetonitrile (MeCN) and methanol (MeOH).

The decomposition of benomyl in organic solvents, acetonitrile and methanol, was also studied by Singh *et al.* [14] using a RP-HPLC method. The RP-HPLC method also confirmed the reversibility of benomyl decomposition to MBC and BIC in acetonitrile. After the decomposition reaction attained the equilibrium, a constant peak height of benomyl was obtained by RP-HPLC analysis of the benomyl solution in acetonitrile for several days. In methanol, however, the decomposition of benomyl to MBC was almost quantitative. These results are shown in Fig. 5. The RP-HPLC results also confirmed the observations reported by Calmon and Sayag [17], *i.e.*, the decomposition of benomyl in organic solvents was slowed down by the addition of water in the reaction medium. The observed rate constant (k) for the forward decomposition reaction of benomyl to MBC in pure methanol at $2.5 \cdot 10^{-4}$ s^{-1} decreased to $6.2 \cdot 10^{-5}$ s^{-1} in methanol–pH 7 phosphate buffer (50:50, v/v).

3. REVIEW OF CHROMATOGRAPHIC METHODS

It is obvious from the above-mentioned kinetic results that benomyl, once dissolved in solutions, is not stable, either in an aqueous medium or in common organic solvents. As a result, for the determination of benomyl in different media, it is first converted to a stable compound which was then determined using chromatographic methods, most notably, thin-layer chromatography (TLC), gas chro-

matography (GC) and HPLC. A brief description of chromatographic methods reported for the determination of benomyl in water, wettable powder (WP) formulations and crops by TLC, GC and HPLC methods is presented in the following paragraphs.

3.1. TLC methods

Benomyl and its degradation products MBC and 2-AB were separated on TLC sheets coated with silica gel (100 μm). The coated material contained a fluorescent indicator [19]. Two-dimensional solvent systems containing benzene–methanol (9:1) and ethyl acetate–chloroform (6:4) were used to obtain the best separation of benomyl, MBC, 2-AB, thiophanate methyl, and benzimidazole. The detection of the compounds on TLC plates was carried out under 254 nm UV light as dark spot. A chromogenic spray reagent (0.5% solution of N-2,6-trichloro-*p*-benzoquinoneimine) was used for selective determination of MBC. The two dimensional TLC offered excellent accuracy and better separation than those developed in one dimension only.

White and Kilgore [20] used TLC for the determination of benomyl (as MBC) and MBC in food crops such as apple, apricot, cherry, grape, nectarine, peach, and plum treated with benomyl. In this method freshly prepared aliquots of benomyl solution were added to fruit-control macerates prior to extraction. The compound was first extracted with benzene and partitioned into 0.1 mol/l hydrochloric acid. The acidic layer was washed several times with chloroform and then neutralized to pH 7.8–8.2 with concentrated sodium hydroxide. During the extraction procedure all benomyl converted to single residual product, MBC, which was then partitioned into ethyl acetate. The compound was concentrated by evaporation of ethyl acetate and subsequently developed on a commercially prepared TLC sheets (polyamide precoated plastic sheets containing a fluorescent indicator). The TLC sheets were first developed in a mixed solvent system containing chloroform–ethyl acetate–acetic acid (190:10:4) to eliminate the streaking of MBC. On dry sheets samples were spotted (in 0.5 cm diameter) approximately 3 cm apart along an imaginary line, 2 cm from the bottom of the TLC sheet. A standard solution of MBC was spotted on each TLC plate to serve as a reference. Separation was achieved in a mixed solvent, chloroform–ethyl acetate–acetic acid (190:10:4) and the spots were identified under UV light (2537 Å). The spots corresponding to MBC R_F values were extracted from the TLC plate with a vacuum-assisted spot collector and the compounds were eluted from the TLC support medium with absolute methanol. Quantitative determination of extracted MBC was carried out spectrophotometry at 287 nm utilizing semimicro quartz cells having 10-cm light paths for residues with MBC concentrations lower than 0.2 mg/l. For residues containing large concentrations of MBC, a 1-cm quartz cell was used.

The lower limit of detection for this method was reported at 0.05 mg/l. Overall average recovery of benomyl residues (determined as MBC), obtained from fortified control samples was, 87%.

In another TLC method, Baker *et al.* [21] used aluminium oxide F_{254} neutral (type E) and silica gel 60 F_{254} coated TLC plates. The separation of benomyl and MBC from six other fungicides was achieved in the following four solvent systems: diethyl ether–glacial acetic acid–methanol (100:5:2), acetone (100%), light petroleum (b.p. 60–80°C)–acetone (3:1), and diethyl ether–methanol (40:1). The fungicides were visualized under UV light or by spraying potassium iodobismuthate solution followed by exposure to bromine vapour. The detection limit for benomyl determination was estimated at 0.8 μg.

It is obvious from the above that TLC can be used as a simple method for the separation of benomyl (MBC) from coextractives obtained from water, WP formulations, and crops. As reported by White and Kilgore [20], TLC can also be used for the quantitative determination of benomyl (MBC). However, the limitation of the TLC method, where benomyl is determined as MBC, is the overestimation of benomyl quantity. Being a natural degradation product of benomyl, MBC is always present in the samples with benomyl, and determined as benomyl (in the methods where benomyl is determined as MBC). This results in a positive error in the determination of benomyl. Another problem with this method is that it cannot be used for the determination of the fate of benomyl after its spray on crops. Baude *et al.* [22] have reported the only TLC method where determination of intact concentrations of

benomyl and MBC in crops was made. In this method crop tissues containing [^{14}C]benomyl were first macerated in 1 mol/l NaOH. The mixture was immediately refluxed. As a result all the [^{14}C]benomyl was converted into [^{14}C]BBU. Any concentration of [^{14}C]BBU which may be present in the crop tissues was transformed into [^{14}C]2-AB. After the reflux step, it was possible to analyze aliquots of sodium hydroxide solution directly by TLC, using ^{14}C-readout techniques. Quantitative determinations were made by scraping TLC spots corresponding to [^{14}C]BBU and [^{14}C]2-AB, and their subsequent liquid scintillation counting. However, when green plant tissue samples were involved, extraction of BBU and 2-AB from refluxed solution was necessary before TLC analysis could be performed. The extraction of BBU and 2-AB in the refluxed solutions was carried out with ethyl acetate. Hexane washes of the basic solutions, prior to ethyl acetate extraction, were sometimes used as an additional clean-up step.

3.2. GC methods

Rouchaud and Decallone [23] developed a GC method for the analysis of MBC in plants and soil. Benomyl and MBC were extracted from melon plants and soil using an extraction method similar to that reported by Kirkland [24] (described under HPLC methods). Residual benomyl and MBC were extracted with benzene and partitioned into 0.1 mol/l HCl. The acidic layer was washed several times with chloroform and then neutralized. The single residual product MBC (present initially in the plant and soil, and formed during the acidic clean-up by the quantitative hydrolysis of benomyl) is partitioned into ethyl acetate. MBC was then derivatized into MBC–trifluoroacetylate (MBC–TFA) for its determination by GC, using ^{3}H electron-capture detection (ECD). A glass column, 1.5 m × 2.2 mm I.D. packed with 5% SE-30 on 80–100 mesh Chromosorb, was used for the separations of desired analytes. For MBC-TFA determination, column temperature was maintained at 140°C. A volume of 1.5 μl of residue was injected into the injector, set at 250°C. The temperature of detector was set at 225°C. Nitrogen, at 40 ml/min, was used as carrier gas. After 10 injections (one day's work) the column temperature was raised to 210°C during the night in order to clean the column from high boiling natural products, unrelated to MBC. The overall recovery of benomyl residues obtained from fortified control samples ranged from 80 to 100%. The minimum detectable concentration of the method was estimated at 0.02 mg/l.

Pyysalo [25] described a modification of the Rouchaud and Decallonne [23] method. In this modified method MBC is derivatized into mainly 2-AB acetate which was determined by capillary GC with nitrogen–phosphorus selective and electron-capture detectors. A 50-m long glass capillary column (0.25 mm I.D.) coated with OV-101 liquid phase was used for achieving the desired separations. Hydrogen was used as carrier gas with the splitless injection technique. Sensitivity of this method at 0.00001 mg/l (for benomyl as MBC) was a significant improvement over the Rouchaud and Decallonne [23] method. The method was tested for various vegetables and fruits.

Cline *et al.* [26] also used a GC method (with ECD) for the determination of very small concentrations of MBC in black walnut fruits by derivatizing MBC into a pentafluorobenzyl bromide derivative. Other chromatographic conditions of this method were similar to that used in the Rouchaud and Decallonne [23] method.

3.3. HPLC methods

The HPLC methods, developed for the determination of benomyl, can be divided into six categories. A brief description of these methods is presented in the following sections.

3.3.1. Determination of benomyl as MBC, after its quantitative conversion to MBC

The first HPLC method based on this approach was reported by Kirkland [24]. In this method benomyl was first quantitatively converted to MBC, by hydrolyzing the sample in aqueous acid. The compounds of interest were then extracted from the acidic solution by organic solvents. The extract was cleaned up by a solvent–solvent partitioning process, and the compounds were determined by high-speed strong cation-exchange liquid chromatography, using a 1000 mm × 2.1 mm I.D. stainless-steel column containing Zipax SCX strong cation-exchange packing. The eluent, a mixture of 0.15

mol/l each of sodium acetate and acitic acid mixed in a 7:3 ratio, was used at a flow-rate of 0.5 ml/min. The column temperature was maintained at 60°C. A highly sensitive ultraviolet (UV) photometer operating at 254 nm was used as a detector. The method was used to determine benomyl in cow milk, urine and tissues. The recoveries of benomyl and its metabolites (other than MBC) averaged about 80% in cow milk and urine. Lower benomyl recoveries (50–80%) were obtained from tissue samples and feces. The minimum detectable concentrations for benomyl/MBC were 0.02 mg/l in milk, and 0.01 mg/l in urine, feces and cow tissues.

The same method with slight modification in the extraction step was used by Kirkland *et al.* [27] for the determination of benomyl in soils and plant tissues. In the modified method any 2-AB, present in the samples, was also extracted and simutaneously determined with MBC. Recoveries of benomyl (as MBC), MBC and 2-AB from various types of soils averaged 92, 88, and 71%, respectively. The minimum detectable concentration of benomyl, MBC and 2-AB was estimated at 0.05 mg/l.

Spittler *et al.* [28], using similar extraction and chromatographic procedures as described by Kirkland [24], determined benomyl (as MBC) and 2-AB in water, brussels sprouts, snap beans, grapes, endive, bok choy, cauliflower, and beet tops. Detection limits for benomyl in the commodities ranged from 0.002 to 0.02 mg/l. Recoveries for MBC ranged between 75 and 114%. Recoveries for 2-AB at 57 to 67% in crops were significantly lower, as compared to MBC.

Zweig and Gao [29] determined benomyl, after its quantitative conversion to MBC in acetonitrile. These authors mentioned that a waiting period of 3 h, after the extraction of benomyl in acetonitrile, ensured the quantitative conversion of benomyl to MBC, at room temperature. RP-HPLC with a RP-18 Spheri 5, Brownlee Labs. bonded reversed-phase column (25 cm × 2 mm I.D.) was used for the determination of MBC. The eluent, a mixture of aceonitrile–water (50:50, v/v) was used at a flow-rate of 1.5 ml/min. Peak detection was made by a UV detector. The recoveries of added MBC and benomyl from surgical gauze patches ranged from 87.0 to 100.4%.

RP-HPLC was also used by Farrow *et al.* [30] [Spherisorb ODS packed into a 150 mm × 4.6 mm I.D. stainless-steel column; eluent methanol–water–ammonia (60:40:0.6, v/v/v); flow-rate, 1 ml/min; UV detector] and Sanchez-Brunete *et al.* [31] [RP-18, 5 μm phase into a 250 × 4.6 mm I.D. stainless-steel column; eluent, methanol–0.3% ammonia (60:40, v/v) flow-rate, 1 ml/min; UV detection] for the determination of benomyl as MBC. The determination was made after quantitative conversion of benomyl to MBC during acid hydrolysis and extraction in organic solvents from crops. Kiigemagi *et al.* [32] employed HPLC with UV detection for the determination of benomyl (as MBC) residues in postharvest-treated pears in cold storage. A CN column (10 cm × 5 mm I.D., 10 μm μBondapak) was used for the separation and determination of MBC in pears. A tetrahydrofuran–water mixture (60:40, v/v) was used as mobile phase at 1.5 ml/min flow-rate. Their clean-up procedure included the extraction of benomyl (MBC) in acetone from acidified pear puree followed by partitioning with ethyl acetate.

A Diol column (LiChrosorb Diol, 5 μm, 15 cm × 4.6 mm I.D.), a mixture of hexane and isopropanol as mobile phase, and UV detection at 285 nm was used by Bicchi *et al.* [33] for the separation and determination of MBC. Their method is based on a clean-up procedure carried out on an Extrelut 20 cartridge. The average recovery for MBC in apples, pears and their pulps was reported at 83.8%.

Liu *et al.* [7] reported a HPLC–mass spectrometry–selected ion monitoring method for the determination of benomyl after its quantitative conversion to MBC. These authors used a 25 cm × 4.6 mm I.D. Whatman Partisil 5 ODS-3 column with a particle size of 5 μm, and a mixture of acetonitrile–0.1 mol/l ammonium acetate (85:15, v/v) as mobile phase running at 1 ml/min flow-rate for the separation of MBC in fruits and vegetables. The mass spectrometer was operated in the positive ion filament mode and selected ion monitoring of m/z 192 was performed. The method was tested for the determination of benomyl in tomatoes, peaches and apples. The minimum detectable level of benomyl in apples, peaches, and tomatoes was estimated at 0.025 mg/l. Recoveries of fortified benomyl at 0.1 mg/l were in the range 85 to 110% in all three commodities.

3.3.2. Determination of benomyl as benomyl after its stabilization in organic solvents by BIC

Determination of benomyl as benomyl in wettable powder (WP) formulations by RP-HPLC has been reported by Stringham and Teubert [34]. In this method WP formulations were extracted with acetonitrile that contained 3% *n*-butyl isocyanate (BIC), and chromatographed on a reversed-phase C_{18} column. This method is based on the finding by Chiba and Cherniak [18] who reported that the degradation of benomyl to MBC in most organic solvents is reversible *i.e.*, benomyl (k_{21}) ↔ MBC + BIC (k_{12}), as described earlier in this review. The method showed good reproducibility with relative standard deviation (R.S.D.) of 1.95%. Mean recovery of standard from the sample pool was estimated at 95% with a standard deviation of 3.50%. A collaborative study of this method was also carried out [35]. The collaborators stated the method as simple, rapid, and reproducible. Statistical analysis of analytical data showed the method to be precise and free from expected interferences.

3.3.3. Simultaneous determination of benomyl and MBC after selective conversion of MBC to MBC–PIC (n-propyl isocyanate) and stabilization of benomyl by the addition of excess BIC

Chiba and Veres [36] developed a method for the determination of residual benomyl and MBC on apple foliage. Samples leaves, in a Mason jar, were freeze-dried and tumbled for extraction in $CHCl_3$ containing 5000 μg *n*-propyl isocyanate/ml at 1°C. During this treatment free MBC present in the sample was quantitatively converted to methyl 1-(*n*-propylcarbamoyl)-2-benzimidazole carbamate (MBC–PIC). *n*-Butyl isocyanate was added to the extract at 5000 μg/ml to prevent the degradation of benomyl during HPLC analysis. A volume of 20 μl of this mixture was injected onto the column, a Brownlee LiChrosorb silica gel column with a guard column in series. The mobile phase contained a mixture of chloroform–hexane (4:1, v/v) that was saturated with water. MBC–PIC, formed from MBC, was separated from benomyl and simultaneously determined. The detection limit for both the compounds in apple leaves was estimated at 0.2 mg/l.

3.3.4. Simultaneous determination of benomyl and MBC after quantitative conversion of benomyl to STB (STB method)

A HPLC method was developed by Chiba and Singh [37] for the simultaneous determination of benomyl and MBC in water and WP formulations. In the method benomyl was converted to 3-butyl-2,4-dioxo-*s*-triazino[1,2-*a*]benzimidazole (STB) in 0.125 mol/l sodium hydroxide. After the conversion (at room temperature in 20 min), STB was determined simultaneously with MBC (which remained intact with the sodium hydroxide treatment) at 286 nm after HPLC separation on a C_{18} column (15 cm × 4.6 mm I.D., Regis HiChrom reversible, 5-μm Spherisorb ODS or ODS-II). A mixture of acetonitrile–water–phosphate buffer (pH 7) (40:45:15, v/v) was used as the mobile phase at flow-rate of 0.8 ml/min. The resolution of STB and MBC was influenced by the composition and pH of the mobile phase, and also by the composition of sample solutions prepared for HPLC injections. To obtain a good resolution between STB and MBC, the factors to be considered were the percentages of organic solvents (acetonitrile), pH and buffer concentration [37] in the mobile phase. The method worked well for the analysis of samples containing varying concentrations of benomyl and MBC. The method was also applied for the determination of benomyl and MBC in WP formulations [38], organic solvents [14], and pathological samples [3]. The method was also used for the determination of benomyl, MBC, STB and BBU in aqueous solutions of different pH values, saturated with benomyl [8].

The conversion of benomyl to STB (the conversion on which this method is based) was found to be quite selective. The method was found accurate and showed good reproducibility with R.S.D. of 0.7% for benomyl and 2.2% for MBC determinations, respectively in WP formulations.

3.3.5. Determination of benomyl and MBC after their respective conversions to BBU and 2-AB

Chiba [39] reported a method for the determination of benomyl and MBC in apples. In this method benomyl and MBC were quantitatively converted to BBU and 2-AB, respectively. Macerated apples were mixed with sodium hydroxide solution (pH 13.2) and the mixture was refluxed for 2 h at 100°C.

During this high-temperature alkali treatment, benomyl quantitatively converted to BBU, and MBC converted to 2-AB. The converted compounds were extracted in diethyl ether–2-butanol (50:50, v/v). The residue, obtained after evaporation of the extractant, was dissolved in a solvent mixture containing methanol–water (30:70). Determinations were made by HPLC with UV detection at 280 nm. BBU and 2-AB were separated from coextractives on a short size-exclusion column connected to a CN analytical column via a switching valve. Separation of BBU and 2-AB was achieved on the CN column.

Maeda and Tsuji [40] determined benomyl in plant tissue by HPLC (Hitachi Gel No. 3010-CH_2OH, 20–25 μm particle size, 500 × 2.1 mm I.D. column; column temperature 45°C; methanol containing 0.1% acetic acid as the mobile phase; flow-rate 1.2 ml/min) after quantitative conversion of benomyl to 2-AB (via MBC). Recoveries of benomyl were reported at 90.5–102.9% with no interference from plant tissue components. The method can detect up to 0.02 mg/l benomyl.

3.3.6. Direct determination of benomyl

Benomyl can be separated in its intact form from other pesticides and its degradation products by HPLC, as reported by Austin *et al.* [41] [Zorbax-silica column and propan-2-ol–hexane (5:95, v/v) at flow-rate of 0.25 ml/min; UV detection], Cabras *et al.* [42] (C_{18} column at temperature 20–50°C; mixtures of water, acetonitrile and phosphate buffer as mobile phase; UV detection at 221.0 nm), Singh and Chiba [8] [Regis Hi-Chrom reversible, 5-μm Spherisorb, 15 cm × 4.6 mm I.D.; mixtures of acetonitrile–water–phosphate buffer (pH 7) as mobile phase; UV detection at 286 nm], Chiba and Northover [43] and Northover and Chiba [44] [Regis Hi-Chrom reversible ODS-1, 5-μm Spherisorb, 15 cm × 4.6 mm I.D. column; mixture of methanol–0.034 mol/l phosphate buffer, pH 7.0 (40:60, v/v) at flow-rate 0.5 ml/min; UV detection at 286 and 294 nm], Singh *et al.* [14] (Regis Hi-Chrom reversible, 5-μm Sperisorb 15 cm × 4.6 mm I.D., and a Phenomex ODS-2 (C_{18}) 15 cm × 4.6 mm I.D.; mixtures of acetonitrile–water–phosphate buffer (pH 7) as mobile phase; flow-rates 0.8–1.5 ml/min; UV detection at 220 and 286 nm), and Marvin *et al.* [45,46] (3 cm × 4.6 mm I.D. C_8 precolumn; acetonitrile–water gradient mobile phase at flow-rates 1–1.5 ml/min; UV detection at 220 nm). However, due to the unavailability of benomyl stock solutions of desired strength in aqueous media at room temperature, it was not possible to exploit the HPLC separation of benomyl for its direct analytical determination until recently. Nevertheless, direct determination of benomyl by HPLC made it possible to study the kinetics of benomyl degradation in solutions [14]. The method was also used for the study of biological activity of benomyl [43,44].

4. RECENT DEVELOPMENTS

Recently, Singh *et al.* [47] reported that preparation of stable stock solutions of desired strength of benomyl in aqueous media is possible. Therefore, direct determination of intact concentrations of benomyl (and MBC) can accurately be made now. Actually, the decomposition of benomyl in water and in the mixed water and organic solvents is not very fast as reported (and believed by many researchers) earlier. According to the decomposition kinetics results [14], approximately 2% benomyl will decompose to carbendazim in 5 min in a solution containing acetonitrile–water (60:40). This suggested that during chromatographic determination of benomyl, the decomposition of benomyl to MBC will not be significant enough to introduce large error in its analytical determination. Preliminary results of direct quantitative analysis of benomyl by HPLC and fast-atom bombardment mass spectrometry (FAB-MS) have recently been reported by Singh *et al.* [48].

5. CRITICAL COMMENTS AND CONCLUDING REMARKS

It is clear from the above that majority of the methods used for the analytical determination of benomyl are based on quantitative conversion of benomyl to its degradation compound, MBC which is determined by HPLC, GC or TLC techniques. The principal drawback of these methods is the over-estimation of benomyl with a wide range of errors. The over-estimation of benomyl is due to the fact that MBC, which is a natural degradation product of benomyl, is present in different samples with benomyl, and is also determined as benomyl. MBC is also a known degradation compound of thiophanate-methyl, a fungicide not as widely used

as benomyl but registered for use on some fruits and vegetables. Therefore, determination of benomyl as MBC would be subjected to positive errors. Moreover, since MBC is also fungitoxic and its level of activity is different from that of benomyl these compounds should be determined individually, particularly for pathological studies. For these reasons methods based on the determination of benomyl as MBC have only limited application.

We would also like te mention that in most of these methods, acid hydrolysis of benomyl is believed to be causing the conversion of benomyl to MBC. However, in our recent work we found that the presence of acid in solution actually stabilized benomyl. Protonation of benomyl at the nitrogen of benzimidazole ring is believed to be responsible for the stabilization of benomyl. This contrasts with the findings of many researchers who reported to achieve a quantitative conversion of benomyl to MBC in acidic solutions with the presence of some organic solvents. We suspect that in these methods temperature [49], duration of time, and presence of organic solvents have caused the conversion of benomyl to MBC and not the presence of acid.

It is also to be pointed out that in common organic solvents (except in methanol and ethanol) quantitative conversion of benomyl to MBC is not possible, at room temperature [18]. This is due to the reversible nature of the decomposition reaction of benomyl to MBC and BIC in organic solvents, as mentioned earlier in this review. This contrasted with the report made by Zweig and Gao [29], who claimed to have achieved quantitative conversion of benomyl to MBC in acetonitrile, at room temperature in three hours.

The method reported by Stringham and Teubert [34] and Teubert and Stringham [35], where benomyl and MBC are determined as benomyl, is also subjected to over-estimation of benomyl. In this method wettable powder formularions were extracted in acetonitrile that contained 3% BIC. Excess amount of BIC was added in the solution to stabilize benomyl. However, excess amount of BIC in solution will also produce benomyl from MBC, which being a natural degradation compound of benomyl, is always present at variable quantities in any kind of benomyl containing samples.

The over-estimation of benomyl by the method of Stringham and Teubert may be explained on the

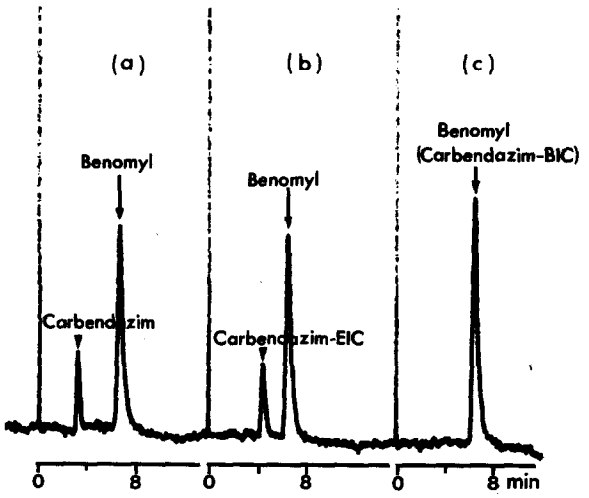

Fig. 6. Chromatograms of MBC, benomyl and MBC-EIC (ethyl isocyanate). Column: 15 cm × 4.6 mm I.D. Regis ODS, mobile phase: acetonitrile–water–buffer (60:30:10). (a) Chromatogram of 50% WP sample dissolved in cold acetonitrile, showing MBC and benomyl peaks; (b) chromatogram of 50% WP sample dissolved in cold acetonitrile in which 2% (v/v) EIC was added, showing the peaks of MBC–EIC and benomyl; and (c) chromatogram of benomyl after 50% WP formulation was dissolved in acetonitrile containing 1000 mg/l of BIC showing benomyl peak only.

basis of Fig. 6a and c, which show the chromatograms of a WP formulation, dissolved in cold (1°C) acetonitrile in the absence and presence of excess BIC. The presence of chromatographic peaks due to MBC and benomyl in Fig. 6a reveals that both of these compounds were present in the WP formulation (as benomyl does not decompose to MBC in cold acetonitrile). However, in excess of BIC, only one peak due to benomyl was observed (Fig. 6c), due to the conversion of MBC to benomyl. The benomyl peak in Fig. 6c was approximately 20% higher than benomyl peak in Fig. 6a. Concentrations of MBC and intact benomyl in WP formulation can be accurately determined using a method similar to that reported by Chiba and Veres [36] (Fig. 6b) or by the STB method [37]. Although chromatographic methods, especially HPLC methods, are more popular for the determination of benomyl, the spectrophotometric method reported by Chiba [50,51] can also be used for the determination of intact concentrations of benomyl and MBC in WP formulations. The determination of MBC and

intact concentrations of benomyl in crops can be carried out by the methods of Baude *et al.* [22] and Chiba [39].

REFERENCES

1 *The Biological and Economical Assessment of Benomyl*, United States Department of Agriculture, Technical Bulletin No. 1678, November, 1985.
2 C. J. Delp, in H. Lyr (Editor), *Modern Selective Fungicides — Properties, Applications, Mechanisms of Action*, Longman Group, London, 1987, pp. 233–244.
3 M. Chiba, A. W. Bown and D. Danic, *Can. J. Microbiol.*, 33 (1987) 157.
4 D. J. Hall, *Proc. Fla. State Hortic. Soc.*, 93 (1980) 341.
5 R. S. Hammerschlag and H. D. Sisler, *Pestic. Biochem. Physiol.*, 3 (1973) 42.
6 W. Koller, C. R. Allen and P. E. Kolattukudy, *Pestic. Biochem. Physiol.*, 18 (1982) 15.
7 C. H. Liu, G. C. Mattern, X. Yu and D. Rosen, *J. Agric. Food Chem.*, 38 (1990) 167.
8 R. P. Singh and M. Chiba, *J. Agric. Food Chem.*, 33 (1985) 63.
9 P. Slade, *J. Assoc. Off. Anal. Chem.*, 58 (1975) 1244.
10 S. Gorbach, *Pure Appl. Chem.*, 52 (1980) 2569.
11 J. P. Calmon and D. R. Sayag, *J. Agric. Food Chem.*, 24 (1976) 311.
12 J. P. Calmon and D. R. Sayag, *J. Agric. Food Chem.*, 24 (1976) 314.
13 E. R. White, E. A. Bose, J. M. Ogawa, B. T. Manji and W. W. Kilgore, *J. Agric. Food Chem.*, 21 (1973) 616.
14 R. P. Singh, I. D. Brindle, C. D. Hall and M. Chiba, *J. Agric. Food Chem.*, 38 (1990) 1758.
15 R. P. Singh and M. Chiba, unpublished results.
16 M. Chiba and F. Doornbos, *Bull. Environ. Contamin. Toxicol.*, 11 (1974) 273.
17 J. P. Calmon and D. R. Sayag, *J. Agric. Food Chem.*, 24 (1976) 426.
18 M. Chiba and E. A. Cherniak, *J. Agric. Food Chem.*, 26 (1978) 573.
19 F. G. von Stryk, *J. Chromatogr.*, 72 (1972) 410.
20 E. R. White and W. W. Kilgore, *J. Agric. Food Chem.*, 20 (1972) 1230.
21 P. B. Baker, J. E. Farrow and R. A. Hoodless, *J. Chromatogr.*, 81 (1973) 174.
22 F. J. Baude, J. A. Gardiner and J. Y. C. Han, *J. Agric. Food Chem.*, 21 (1973) 1084.
23 J. P. Rouchaud and J. R. Decallonne, *J. Agric. Food Chem.*, 22 (1974) 259.
24 J. J. Kirkland, *J. Agric. Food Chem.*, 21 (1973) 171.
25 H. Pyysalo, *J. Agric. Food Chem.*, 25 (1977) 975.
26 S. Cline, A. Felsot and L. Wei, *J. Agric. Food Chem.*, 29 (1981) 1087.
27 J. J. Kirkland, R. F. Holt and H. L. Pease, *J. Agric. Food Chem.*, 21 (1973) 368.
28 T. D. Spittler, R. A. Marafioti and L. M. Lahr, *J. Chromatogr.*, 317 (1984) 527.
29 G. Zweig and R. Gao, *Anal. Chem.*, 55 (1983) 1448.
30 J. E. Farrow, R. A. Hoodless, M. Sargent and J. A. Sidwell, *Analyst*, 102 (1977) 752.
31 C. Sanchez-Brunete, A. D. Cal, P. Melgarejo and J. L. Tadeo, *Int. J. Environ. Anal. Chem.*, 37 (1989) 35.
32 U. Kiigemagi, R. D. Inman, W. M. Mellenthin and M. L. Deinzer, *J. Agric. Food Chem.*, 39 (1991) 400.
33 C. Bicchi, F. Belliardo, L. Cantamessa, *Pestic. Sci.*, 25 (1989) 355.
34 R. W. Stringham and W. E. Teubert, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 302.
35 W. E. Teubert and R. W. Stringham, *J. Assoc. Off. Anal. Chem.*, 67 (1984) 303.
36 M. Chiba and D. F. Veres, *J. Assoc. Off. Anal. Chem.*, 63 (1980) 1291.
37 M. Chiba and R. P. Singh, *J. Agric. Food Chem.*, 34 (1986) 108.
38 R. P. Singh and M. Chiba, *J. Assoc. Off. Anal. Chem.*, (1993) in press.
39 M. Chiba, in R. Greenhalgh and T. R. Roberts (Editors), *Proceedings of the Sixth International Congress of Pesticide Chemistry, Ottawa, August 10–15, 1986*, Blackwell, London, 1987, pp. 339–340.
40 M. Maeda and A. Tsuji, *J. Chromatogr.*, 120 (1976) 449.
41 D. J. Austin, K. Alan and I. H. Willimans, *Pestic. Sci.*, 7 (1976) 211.
42 P. Cabras, M. Meloni, M. Perra and F. M. Pirisi, *J. Chromatogr.*, 180 (1979) 184.
43 M. Chiba and J. Northover, *Phytopathology*, 78 (1988) 613.
44 J. Northover and M. Chiba, *J. Agric. Food Chem.*, 37 (1989) 1416.
45 C. H. Marvin, I. D. Brindle, R. P. Singh, C. D. Hall and M. Chiba, *J. Chromatogr.*, 518 (1990) 242.
46 C. H. Marvin, I. D. Brindle, C. D. Hall and M. Chiba, *J. Chromatogr.*, 555 (1991) 147.
47 R. P. Singh, C. H. Marvin, I. D. Brindle, C. D. Hall and M. Chiba, *J. Agric. Food Chem.*, 40 (1992) 1303.
48 R. P. Singh, I. D. Brindle, T. R. B. Jones, J. M. Miller and M. Chiba, *Rapid Commun. Mass Spectrom.*, 7 (1993) 167.
49 M. Chiba, *Bull. Environ. Contamin. Toxicol.*, 18 (1977) 285.
50 M. Chiba, *J. Assoc. Off. Anal. Chem.*, 62 (1979) 488.
51 M. Chiba, *J. Agric. Food Chem.*, 25 (1977) 368.