Determination of Organophosphorus Pesticides in Biological Samples of Acute Poisoning by HPLC with Diode–Array Detector

Yuko Cho,*[1] Nancy Matsuoka, and Akira Kamiya

Department of Pharmacy, Yamaguchi University Hospital, 1144 Kogushi, Ube 755, Japan.

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We have developed a simple and rapid method for measuring 11 organophosphorus pesticides (dichlorvos, methidathion, salithion, malathion, fenitrothion, fenthion, parathion, diazinon, ethylthiometon, *O*-ethyl *O*-(4-nitrophenyl)phenylphosphonothioate (EPN) and chlorpyrifos) and one metabolite (3-methyl-4-nitrophenol) of fenitrothion in serum and urine of acute poisoning patients by HPLC with a diode-array detector. An aliquot of the biological sample after deproteinization by acetonitrile was injected into a C_{18} column using acetonitrile–water as a mobile phase. The detection limits in serum and urine ranged from 0.05 to 6.8 μg/ml at a wavelength of 230 nm. This method was successfully applied to two actual cases of acute poisoning.

Key words organophosphorus pesticide; HPLC diode-array; acute poisoning; serum; urine

Organophosphorus pesticides are the compounds causing many cases of acute poisoning. According to statistical data from National Research Institute of Police Science, 1397 accidental poisonings caused by organophosphorus pesticides were reported from 1987 to 1990 in Japan.[2] The number of cases treated in hospitals is assumed to be much higher, because they include suicidal and homicidal poisonings.

The analytical methods of technical and formulated pesticides are well investigated and are summarized in a series of handbooks[3]; however, they are not directly applicable to biological samples taken in an emergency. There is literature on the analysis of some organophosphorus pesticides in urine and plasma by GC,[4] in gastrolavage, blood and urine by GC-MS,[5] in standard mixture, blood, lung and liver by HPLC[6-8] and in blood by LC-MS,[9] but still needed is the developement of a rapid screening and assay method of a series of organophosphates for clinical diagnosis and treatment.

The relationship of organophosphorus pesticide concentration in body fluid (serum and urine) and severity of patient reaction has not yet been well investigated. These data are required to make more certain diagnoses and to anticipate the efficacy of clinical treatment like direct haemoperfusion (DHP) and haemodialysis.[10]

HPLC with a diode-array detector is a nondestructive method and has the advantage of collecting chromatographic data and UV spectral data simultaneously. The usefulness of this means in pesticide analysis has been studied from an environmental point of view.[11,12]

We now describe a qualitative and quantitative method for clinical analysis of organophosphorus pesticides in biological samples of acute poisoning patients using HPLC with a diode-array detector.

Results and Discussion

The compounds were selected from many organophosphates on the basis of statistical data of acute poisoning in Japan from 1987 to 1990[2] and at the request of physicians of the Intensive Care Unit (ICU) in our hospital.

Identification Chromatographic conditions were optimized by using a standard mixture and blank specimen (blood and urine), and changing the flow rate and the concentration of acetonitrile in mobile phase; these were determined as 1.0 ml/min and 50% for eleven compounds (Fig. 1a) and 35% for 3-methyl-4-nitrophenol (Fig. 2a). Figure 1a shows only nine peaks instead of eleven, because methidathion overlapped with salithion, and fenthion overlapped with parathion. However, they have different patterns of UV absorbance, as shown in Fig. 1b—e and can easily be differentiated from each other. Practically, their multiple ingestion is not likely.

Although six compounds in the eleven organophosphorus pesticides have no absorption maximum, each spectrum shows its characteristic pattern, which is useful to distinguish them from each other.

These data on retention time and UV spectra of standards are registered in the library of the computer.

An identification test was made of spiked samples (blood and urine, *ca.* 0.45 μg each). For all peaks the correct references were retrieved with "match" value (max. 1000) from 875 (salithion in blood and urine) to 996 (fenitrothion in blood and *O*-ethyl *O*-(4-nitrophenyl)phenylphosphonothioate (EPN) in blood and urine). In overlapping peaks the ascending and descending parts of the peaks were selected as the points to be examined.

Quantitative Analysis The detection limit at a wavelength of 206 nm of all compounds in standard solutions was from 14 ng/ml (fenitrothion) to 110 ng/ml (ethylthiometon) at a signal-to-noise ratio of 3.

The quantitativeness of biological samples was examined at a wavelength of 230 nm, because base lines of blank samples were not stable at a wavelength of 206 nm. The detection limits in serum were 6.8 (dichlorvos), 0.20 (methidathion), 0.70 (malathion), 4.0 (salithion), 0.15 (fenitrothion), 0.13 (fenthion), 0.48 (parathion), 0.78 (diazinon), 1.1 (ethylthiometon), 0.25 (EPN), 0.61 (chlorpyrifos) and 0.05 (3-methyl-4-nitrophenol) μg/ml; in urine they were 6.8, 0.3, 0.5, 4.0, 0.15, 0.13, 0.48, 0.78, 1.1, 0.25, 0.61 and 0.05 μg/ml, respectively, at a signal-to-noise ratio of 3. The limits of quantitation (LOQ), examined for 6 compounds, ranged from 0.25 (fenthion, coefficient of variation (CV)=4.4, $n=5$) to 8.0 μg/ml (salithion, CV=3.2, $n=5$) in serum, and from 0.25 (fenthion, CV=2.7,

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* To whom correspondence should be addressed.

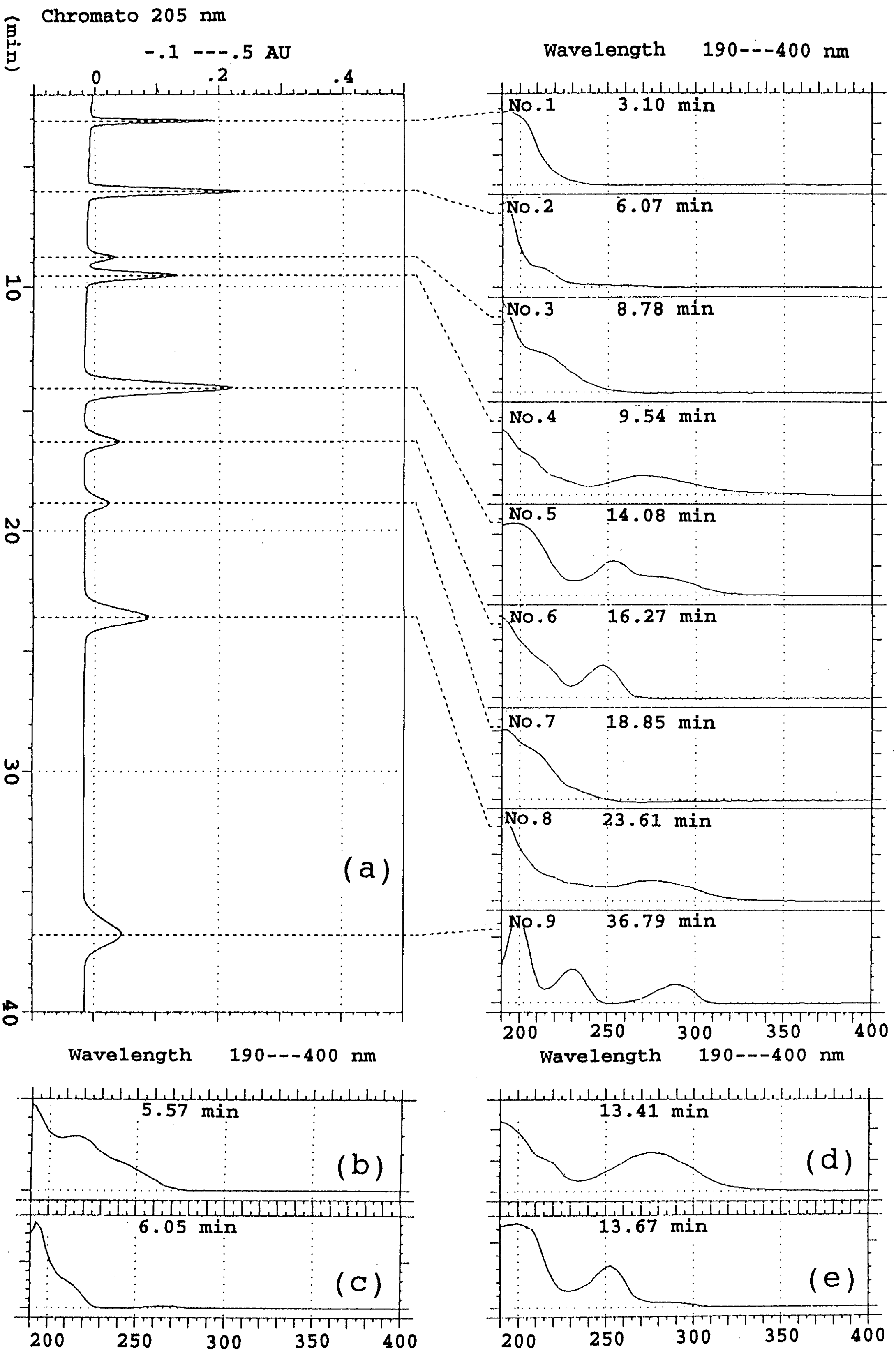

Fig. 1. HPLC Diode-Array UV Chromatograms and UV Spectra of Standards

(a) Column: Nucleosil 5C18, 4 mm × 15 cm. Mobile phase: acetonitrile–water = 50 : 50, v/v. Flow rate: 1.0 ml/min. Peak 1: dichlorvos, peak 2: methidathion and salithion, peak 3: malathion, peak 4: fenitrothion, peak 5: fenthion and parathion, peak 6: diazinon, peak 7: ethylthiometon, peak 8: EPN, peak 9: chlorpyrifos, *ca.* 0.9 μg each. (b) UV spectra of methidathion, (c) salithion, (d) fenthion and (e) parathion.

$n=5$) to 4.0 μg/ml (salithion, CV = 4.6, $n=5$) in urine.

The calibration curves of fenitrothion and 3-methyl-4-nitrophenol obtained by plotting the peak area ratios of each compound to internal standard (I.S.) (10 μg/ml) against the concentrations of each compound from 0.3 (fenitrothion) or 1 (3-methyl-4-nitrophenol) to 100 μg/ml were straight lines. Correlation coefficients of lines were 0.9996 ($n=30$) and 0.9998 ($n=25$), respectively.

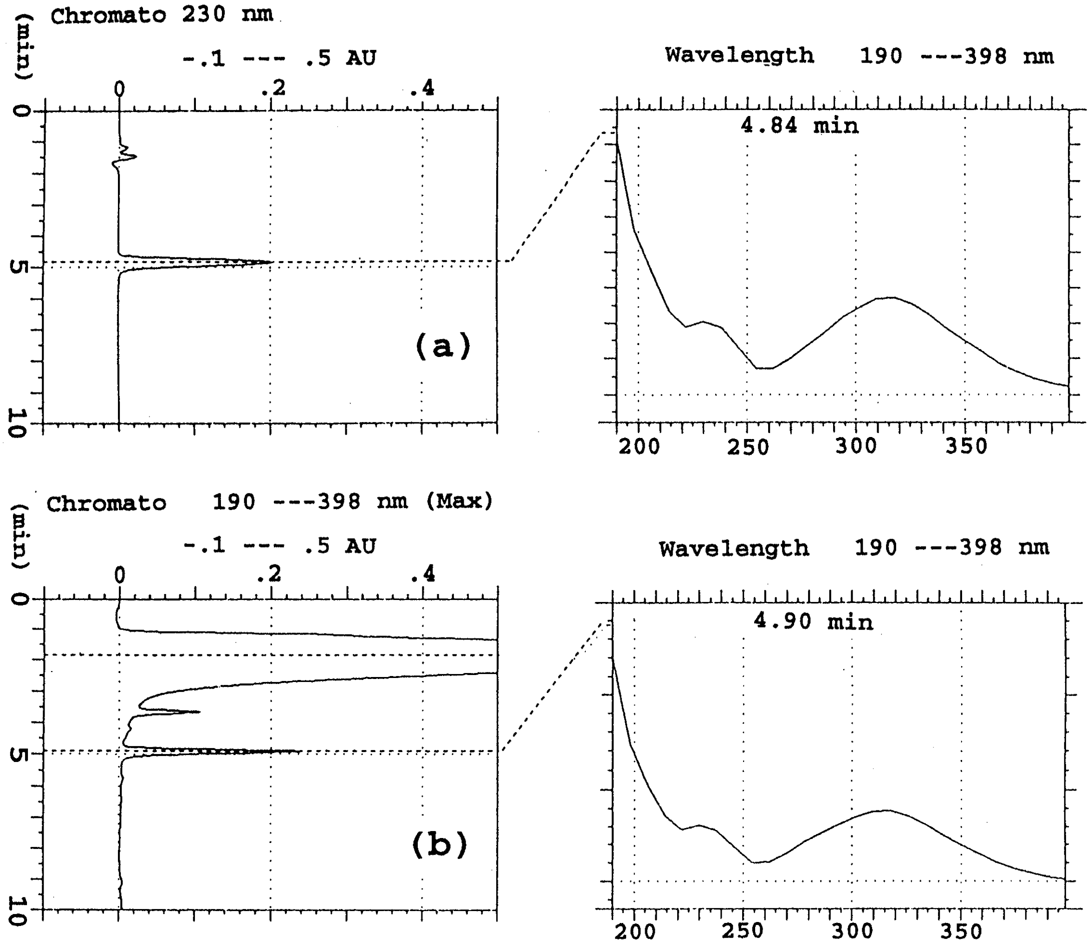

Fig. 2. HPLC Diode-Array UV Chromatograms and UV Spectra of 3-Methyl-4-nitrophenol Standard (a) and Urine Sample of Case 2 (b)

Column: Nucleosil 5C18, 4 mm × 15 cm. Mobile phase: acetonitrile–water = 35 : 65, v/v. Flow rate: 1.0 ml/min. (a) 2.16 μg.

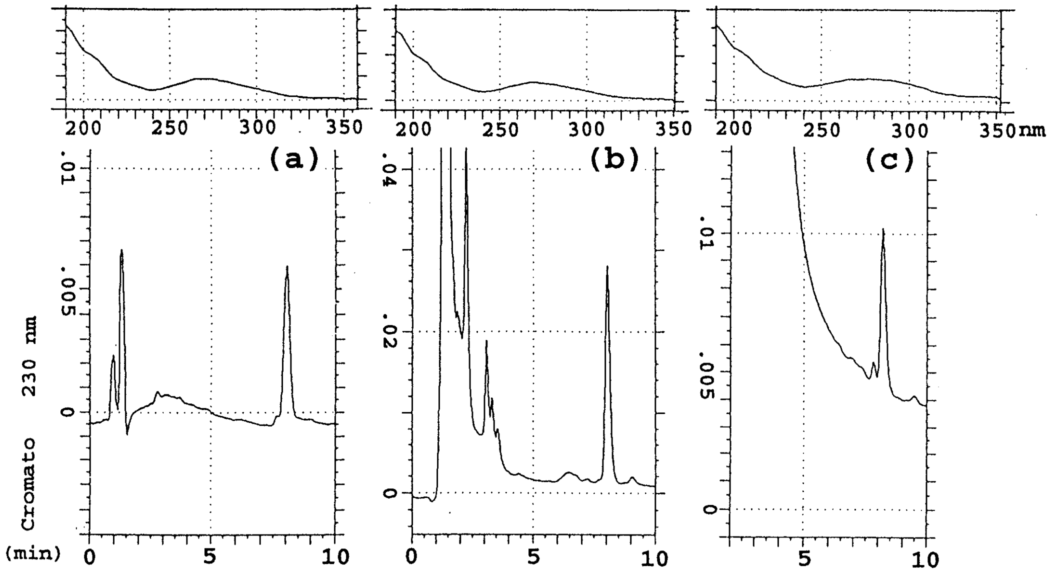

Fig. 3. HPLC Diode-Array UV Chromatograms and UV Spectra of Biological Samples of Case 1

(a) Gastrolavage (diluted to 1/1000), 20 μl (b) serum, 40 μl (c) urine, 200 μl. Same conditions as Fig. 1a.

The recoveries of fenitrothion at the concentrations of 0.3 (LOQ), 10, 100 μg/ml were 97.4%, 98.6% and 99.0% from spiked serum and 101.4%, 101.4% and 101.1% from spiked urine, respectively.

Within-run study (*n* = 5) of samples spiked with 0.3, 10 and 100 μg/ml of fenitrothion gave the mean values of 0.29 (CV = 5.4), 9.86 (CV = 0.64) and 99.0 μg/ml (CV = 0.2) (serum), and 0.30 (CV = 2.9), 10.1 (CV = 0.5) and 101.1 μg/ml (CV = 0.1) (urine). Between-day precision was 0.5—6.5% (serum) and 1.4—3.1% (urine), expressed as CVs

($n=3$) of the amounts given in the concentration range from 0.3 to 100 µg/ml of fenitrothion.

Case Study Case 1 was a female, 66 years old. The information given was that she had gone to a flower shop the previous day to buy something, although it was not clear what she bought, and the ChE value of her serum had become very low (18 IU/l). So fenitrothion, the most frequently used organophosphorus pesticide in Japan, was suspected to be the cause-compound and it was identified in all samples examined (Fig. 3). The concentration of fenitrothion in gastrolavage was 6.1 mg/ml on arrival, 17.1 µg/ml (2 h later, before DHP) and 5.5 µg/ml (7 h later, after DHP) in serum, and 1.8 µg/ml (2 h later) in urine. Analysis of the metabolite, 3-methyl-4-nitrophenol, was not performed in this case, because of a shortage of samples. She was transferred to the general unit from ICU 13 d after admission.

Case 2 was a male, 76 years old, whose anamnesis was aplastic anemia and myocardial infarction. The ChE value on arrival was 19.0 IU/l. This case was a suicide by ingestion of about 50 ml of Sumithion®, a fenitrothion preparation. Fenitrothion was identified in gastrolavage and in some of the serum samples but not in any of the urine samples (2, 10.5 and 24.5 h after ingestion). The concentration of fenitrothion was 557 µg/ml in gastrolavage (2 h after ingestion), 14.5 µg/ml (2 h after ingestion, before DHP) and 4.1 µg/ml (10.5 h after ingestion, after DHP) in serum, but it was not detected in serum 24.5 h after ingestion. The metabolite, 3-methyl-4-nitrophenol, was identified in some urine samples but not in any of the serum samples. The chromatogram of the urine sample (10.5 h after ingestion) and the UV spectrum of the peak due to 3-methyl-4-nitrophenol are shown in Fig. 2b. The concentration of 3-methyl-4-nitrophenol was 29.8 µg/ml (2 h after ingestion, before DHP) and 12.6 µg/ml (10.5 h after ingestion, after DHP) and it was not detected 24.5 h after ingestion. He died of multiple organ failure.

No significant interference on the chromatograms or the UV spectra was observed in either case. According to the literature some of the common drugs may show peaks in a similar retention time range to those of organophosphorus pesticides by this system.[7] However, library search can prevent an error in identification.

The time required to report the first result was 2 h after the arrival of samples in both cases. Thus this method is simple and rapid enough to apply to biological samples in an emergency.

An approach for the measurement of the metabolites and other groups of agricultural chemicals by this system is also being prepared.

Experimental

Materials EPN and parathion were obtained from Gasukuro Kogyo Inc. (Tokyo, Japan), methidathion, ethylthiometon, chlorpyrifos and salithion from Wako (Osaka, Japan), and fenthion and 3-methyl-4-nitrophenol from Hayashi Junyaku (Osaka). Fenitrothion, dichlorvos, malathion and diazinon were generously donated by Kyushu Sankyo (Saga, Japan). HPLC-grade acetonitrile was purchased from Merck (Frankfurt, Germany).

Apparatus HPLC analysis was carried out on a Nucleosil 5C18 column (4 mm × 15 cm, particle size 5 µm) with a prefilter using a Liquid Chromatograph Model 510 (Waters Associates, Tokyo) controlled by an automated gradient controller, equipped with a Waters Intelligent sample processor, WISP Model 712, LC column oven Model 556 (Gasukuro Kogyo, Tokyo) and a Waters 990J photodiode array detector. All apparatuses were connected to a personal computer PC 9801 RX (NEC, Tokyo).

Conditions The mobile phase was acetonitrile–water (50 : 50, v/v) for most compounds, excepting 3-methyl-4-nitrophenol (35 : 65, v/v). The flow rate was 1 ml/min, column temperature was 40 °C and the detection range used was 190—400 nm.

Procedure Five hundred µl of biological samples (blood, serum, urine or gastrolavage) was mixed with 500 µl of acetonitrile or acetonitrile solution of the appropriate I.S. on a vortex mixer for 20 s. After centrifugation (9500 × *g* for 4 min), 20—200 µl (identification) or 20 µl (quantitation) of an aliquot was subjected to HPLC analysis. The I.S.s used for quantitative analysis were fenthion for fenitrothion, and methidathion for 3-methyl-4-nitrophenol.

References

1) Present address: *Faculty of Engineering, Yamaguchi University, 2557 Tokiwadai, Ube 755, Japan.*
2) National Research Institute of Police Science ed., "Annual Case Reports of Drug and Toxic Poisoning in Japan" (in Japanese), Vol. 29—32, Tokyo, 1986—1990.
3) CIPAC Handbook, "Analysis of Technical and Formulated Pesticides," Vol. 1A, compiled by Ashworth R. B., Henriet J., Lovett J. F., Martijn A., 1980; Vol. 1B, compiled by Henriet J., Lovett J. F., Martijn A., Povlsen H. H., 1983; Vol. 1C, compiled by Henriet J., Martijn A., Povlsen H. H., 1985; Vol. D, compiled by Martijn A., Dobrat W., 1988, Collaborative International Pesticides Analytical Council, Hertfordshire, England.
4) Liu J., Suzuki O., Kumazawa T., Seno H., *Forensic Sci. Int.*, **41** 67—72 (1989).
5) Kojima T., Yashiki M., Miyazaki T., Chikasue F., Ohtani M., *Forensic Sci. Int.*, **41**, 245—253 (1989).
6) Tsunoda N., *Kagaku Keisatsu Kenkyusho Hokoku, Hokagakuhen*, **40**, 16—23 (1987).
7) Okuda T., Sameshima I., Nishida M., *Jpn. J. Ther. Drug Monit.*, **7**, 185—192 (1990).
8) Sharma V. K., Jadhav R. K., Rao G. J., Saraf A. K., Chandra H., *Forensic Sci. Int.*, **48**, 21—25 (1990).
9) Kawasaki S., Ueda H., *J. Chromatogr.*, **595**, 193—202 (1992).
10) Hausmann E., Gellhaus H., Reger-Stroka I., Proceedings of the 30th International Meeting, The International Association of Forensic Toxicologists, 1992, p. 196.
11) Boessenkool H.-J., Cleij P., Goewie C. E., Broek H. H., Klooster A., *Mikrochim. Acta*, **2**, 75—92 (1986).
12) Chichila T. M. P., Gilvydis D. M., *J. AOAC Int.*, **76**, 1323—1328 (1993).