DETERMINATION OF N-METHYLCARBAMATES IN FOODS

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SUMMARY

The multiresidue method using multisolvent extraction, SPE cleanup of the extract, HPLC with the use of OPA post-column reaction and fluorescence detection for the determination of N-methylcarbamate pesticides in food products was used.

A matrix solid phase dispersion method of the isolation and extraction of carbamates was alternatively applied. In the introductory study 44 items of the food basket for the Czech Republic were analysed.

In the major part of the studied samples, the considerable part of which was culinary treated, the concentration of the target carbamates was below the limit of the used detection method. In the concentration range of 10–100 μg/kg in the analysed samples of the studied analytes, aldicarb and its metabolites, e.g. methomyl and methiocarb were being found most frequently.

Key words: N-methylcarbamates, food products, extraction, HPLC determination

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INTRODUCTION

Being both highly effective and having a broad spectrum of activity carbamate pesticides are extensively and world wide applied to protect crops and food from the ravages of pests.

They exhibit herbicidic, fungicidic, acaricidic, nematocidic and rodenticidic effects.

The number and quantity of N-methylcarbamate pesticides used in agriculture continue to increase, replacing the more environmentally stable organohalogen pesticides.

Carbamates inhibit acetylcholinesterases, they are toxic for organisms, and they can attack nervous system giving a rise to the genetic damage. Due to their toxic nature and harmful effect on human organism a great attention is given to the monitoring of carbamates and their metabolites in the environment.

Drinking water sources can be contaminated by agricultural runoff. After being applied to foods they can contain residues of both carbamates and their by-products and if harvested too soon after the application the above mentioned residues can remain in the produce.

Both the development of the multiresidual method of N--methylcarbamate pesticides and the determination and monitoring of these compounds in foods of the food basket for the population of the Czech Republic were implemented in the Centre of Hygiene of Food Chains in Brno, National Institute of Public Health in Prague (1).

List of acronyms: GPC–Gel Permeation Chromatography; HPLC–High Performance Liquid Chromatography; LC–Liquid Chromatography; LOD–Limit of Detection; MSPD–Matrix Solid Phase Dispersion; SPE–Solid Phase Extraction; US–EPA–United States Environmetal Protection Agency

On the basis of the data on the reviewed carbamate pesticides occurrence and in consent with the regulations of the Czech Republic, the commodities in which the occurrence of these pesticides would be probable were selected. The commodities were mostly of the plant origin, e.g.: vegetables, fruits, vegetable and fruit products and cereals.

The isolation of carbamate pesticides from the food matrix followed by HPLC assay was performed on eleven analytes specified in US-EPA method 5.3.1 and most frequently mentioned in the literature, e.g. on: aldicarb, aldicarbsulfoxide, aldicarbsulfone, methomyl, oxamyl, 3-hydroxycarbofuran, carbaryl, carbofuran, propoxur, pirimicarb and methiocarb (2–8).

Considering that the ADI values are very low, they range from 0,1 to 1 $\mu g.kg^{-1}$, it was necessary to work out the analytical method, the LOD of which could make the evaluation of the exposure to those carbamate pesticides in foods possible.

PRINCIPLE OF THE DETERMINATION

From the chemical point of view, N-methylcarbamates are derived from carbamic acid. Their characteristic feature is the hydrolyzable N-methylcarbamoyl moiety. The rest of the molecule is structurally different and its character limits subsequent analysis, determination and detection.

Thus, it would be difficult to include all these above mentioned compounds into a multiresidue method using LC with UV detection and to achieve uniformly low detection limit for each analyte.

The N-methylcarbamoyloximes – oxamyl, methomyl and aldicarb have UV extinction coefficients nearly 3–4 orders of magnitude lower than those of the aromatic ring containing

Table 1. *Validation parametres of the method for determining N-methylcabamates in foods*

Analyt	Recovery (%)	SD (µg/kg)	RSD (%)	LOQ (µg/kg)
Aldicarbsulfoxide	92.0	8.6	9.4	0.9
Aldicarbsulfone	95.4	8.1	8.5	0.07
Oxamyl	96.1	7.2	7.5	1.0
Methomyl	94.1	8.6	9.2	4.8
3-hydroxycarbofuran	82.4	7.2	8.8	0.3
Aldicarb	80.1	7.5	9.4	0.7
Propoxur	58.1	3.7	4.4	0.5
Carbofuran	85.0	7.2	8.5	0.9
Carbaryl	82.0	7.4	9.1	0.8
Pirimicarb	88.2	8.5	9.6	0.5
Methiocarb	76.8	7.75	10.1	0.3

SD = standard deviation, RSD = relative standard deviation, LOQ = limit of quantitation. LOQ was estimated via analysis of blank samples.
Recoveries are valid for concentrations up to 400 µg/kg except for oxamyl (200 µg/kg). The validation parameters were obtained by means of EffiValidation 1.0 software.

Table 2. *Concentrations of N-methylcarbamates (µg/kg) in selected food samples*

Samples	AldicarbSO	AldicarbSO2	Oxamyl	Methomyl	3OHCBF	Aldicarb	Propoxur	Carbofuran	Carbaryl	Methiocarb
Syrup	< 0.9	< 0.07	< 1	<4.8	< 0.3	< 0.7	< 0.5	< 0.9	< 0.8	90.42
Oranges	23.8	369.9	< 1	18.5	0.97	1.5	< 0.5	< 0.9	1.2	1.65
Leafy vegetables	38.4	24	42	48	< 0.3	1.8	< 0.5	< 0.9	< 0.8	< 0.3
Subtropical fruits	< 0.9	< 0.07	< 1	< 4.8	< 0.3	10.74	< 0.5	< 0.9	< 0.8	9.48
Juice	< 0.9	< 0.07	< 1	< 4.8	< 0.3	1.14	< 0.5	< 0.9	< 0.8	8.88
Onions and garlic	1.26	1.26	< 1	< 4.8	1.32	0.96	1.44	< 0.9	< 0.8	< 0.3
Stem vegetables	< 0.9	1.23	< 1	154.4	2.15	1.1	< 0.5	< 0.9	< 0.8	1.1
Cereals (other)	1.6	0.3	< 1	< 4.8	< 0.3	0.54	< 0.5	< 0.9	< 0.8	1.35
Wine	< 0.9	< 0.07	< 1	< 4.8	1.3	1.4	1.9	1.5	3.15	1.92
Tea (infusion)	< 0.9	< 0.07	< 1	< 4.8	< 0.3	< 0.7	< 0.5	1.98	2.52	5.64
Coffee (infusion)	11.22	15.72	18.72	< 4.8	828.6	21.24	51.3	67.8	9.18	21.78
Root vegetables	1.224	< 0.07	< 1	< 4.8	3.36	3	< 0.5	< 0.9	1.56	171.84

AldicarbSO = aldicarbsulfoxide, AldicarbSO2 = aldicarbsulfone, 3OHCBF = 3-hydroxycarbofuran

N-methylcarbamates – propoxur, carbofuran, carbaryl and methiocarb.

As each carbamate elutes from the reverse-phase liquid chromatographic column, it is hydrolysed by strong aqueous base (NaOH) at elevated temperature to release an alcohol, carbon dioxide and methylamine. In the second, i.e. derivatisation step, the methylamine combines with ortho-phtaldialdehyde (OPA) and the nucleophilic 2-mercaptoethanol to form a highly fluorescent isoindole derivative. Thus, this post-column derivatisation method yields a single analyte from ten different compounds and, when coupled with highly sensitive fluorescence detection, provides a mean to measure ppb levels of all analytes.

After chromatographic separation, the N-methylcarbamates were hydrolysed on line with NaOH to methylamine, which was subsequently derivatised with the ortho-phtaldialdehyde and the nucleophilic 2-mercaptoethanol reagents. The fluorophore was then detected very selectively and sensitively with a fluorescence detector (9).

EXPERIMENTAL PART

Samples

Food samples predominantly of the plant origin were collected in the shop network in 12 places of the Czech Republic within the framework of the project "Monitoring of human dietary exposure to chemical contaminants in food chains" (10). The samples were culinary treated and homogenised.

Reagents and Equipments Used

Acetone, dichloromethane, petroleum ether, acetonitrile, methanol, sodium hydroxide, mercaptoethanol, o-phtaldialdehyde, sodium tetraborate decahydrate (Sigma Aldrich), carbamate standards (Dr. Ehrenstorfer and Hewlett-Packard). The used solvents included HPLC grade, reagents p.a. purity, water was deionised.

SPE cartridges C18 (Supelco), SPE-NH2 cartridges a Bond Elut SPE columns and sorbent EnvirElutTM (Varian). Carbamate chromatographic column (Waters) of 150 x 3,9 mm, 4 µm.

The blender for sample homogenisation and extraction (Po-

lytron, Kinematica), SPE vacuum unit (Burdick-Jackson), water purification system (Barnstead B-pure type D 4511), analytical balances (Sartorius), ultrasonic bath (UC 006 DM 1 Tesla), rotation vacuum evaporator (Buchi), GPC (Waters), liquid chromatograph Waters (modules WatersTM 600 Controller with quaternary solvent delivery system, WatersTM 717 Plus autosampler, WatersTM 474 Scanning Fluorescence Detector, postcolumn derivatisation unit with temperature controller, software used: Millennium 32).

Analytical Methods

Extraction and sample cleanup

a) Solid samples

60 ml of acetone was added to the 30 g of mashed sample and the sample was homogenised 60 s using Polytron 11000 rpm. 60 ml of both dichloromethane and petroleumether were then added and while mixing additional 60 s, the extraction took place. The extract was filtered and aliquot part of 20 ml was evaporated nearly to its dryness using a rotary vacuum evaporator, while the remaining solvent was allowed to evaporate in the air.

Extracts of flour and pastry samples are recommended to centrifuge. Nonpolar plant lipid materials were removed using gel permeation chromatography. The evaporated residue was redissolved in 1 ml dichloromethane and cleaned on the SPE aminopropyl-bonded silica column. The elution of carbamates was proceeded with dichloromethane : methanol (99:1). The eluted fractions were evaporated using a rotary vacuum evaporator nearly to their dryness and the remaining solvent was allowed to evaporate in the air again. The residue was then redissolved in 1 ml of acetonitrile/water mixture (90:10) with the help of an ultrasonic bath and before HPLC analysis filtered through the membrane filter 45 μm.

b) Liquid samples

Carbamates in liquid samples were isolated and concentrated by means of SPE cartridges filled with C18. Various solid-phase materials were evaluated. 10 ml of liquid sample was passed through a C18 cartridge. The column was washed up with water and the preconcentrated analytes were eluted by acetone from the cartridge. The resulting eluate was treated in the same way as the extract from solid samples, i.e. it was evaporated nearly to its dryness, dissolved in dichloromethane and coextractives were removed by SPE-NH_2 cleanup before HPLC analysis.

As an alternative method of the isolation of the carbamate pesticides from plant matrix, the matrix solid-phase dispersion (MSPD) combining the homogenisation of the sample, extraction of analytes and cleanup in one step was developed (11). This requires only small amount of the analysed material and it is especially suitable as a screening assay. 2 g of analysed sample and 2 g of sorbent with bonded phase EnvirElutTM were gently blended in a mortar until the mixture was homogenous. The resultant mixture was transferred into a plastic syringe barrel and eluted using dichloromethane.

HPLC analysis

The final determination was performed by the separation of N--methylcarbamates via high performance liquid chromatography with ternary gradient elution (water-methanol-acetonitril) on the Waters Carbamate Analysis column. After their separation on the chromatographic column (as each carbamate elutes from the reversed-phase chromatographic column) carbamates were hydrolysed by strong aqueous base at 80 ºC and derivatised with o-phtaldialdehyde and 2-mercaptoethanol in borate buffer. Fluorescence detection was performed at 339/445 nm.

The external standard method was used for identification and quantification of the analytes, the validation parameters were evaluated by the means of Effi Validation 1.0 software (12).

For each step of the method and for all types of the analysed food matrices the recovery was tested via analysis samples spiked with target pesticides. The recovery of the whole procedure, limits of detection and quantification of each tested carbamate are shown in Table 1.

Stability test

The stability test was performed on the spiked banana purée which contained the known concentration of target analytes under the identical conditions at which samples were stored, e.g. –20 ºC within 14 weeks. The concentration remained unchanged for 2 weeks and after 3–14 weeks it dropped by 20–60 % depending on the carbamate type.

RESULTS AND DISCUSSION

This introductory study provides us both with the basic information and experience in the analysis of carbamate pesticides in food. We developed a screening method for the determination of carbamate pesticides in food with the limit of detection of 1–5 μg/kg according to the character of the matrix. The procedures of isolation with the regard to the type of matrix (liquid and solid samples) were evaluated for the matrices with high content of plant pigments and interfering coextractives and also for flour and pastries. The content of N-methylcarbamates was checked in the following commodities and composites: bananas, oranges, subtropical fruits, apples, peaches, apricots, stone and pome fruits, grapes, small fruits – berries, stewed fruit, fruit products, infant food – fruit puree, syrup, juice, tomatoes, carrots, cauliflower, stem vegetables, leafy vegetables, forced vegetables, fruiting vegetables, root vegetables, onions and garlic, frozen mixed vegetables, vegetable products, ketchup and mustard, legumes, potatoes, potato products, cakes, biscuits, rolls and French loaf, wholemeal bread, bread, rye rolls, cereals, flour and yeast, dumplings, semolina, tea, coffee, beer, vine, spirits. In the major part of the tested composite samples, the considerable part of which was culinary treated, the concentration levels of the target N-methylcarbamates were found to be below the limit of detection. In the concentration range of 10– 100 μg/kg the most frequently aldicarb and its metabolites, methomyl and methiocarb, were found. The highest concentrations of N-methylcarbamates in selected foods are shown in Table 2.

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