Technical Paper

Validation of the Method for the Determination of Dithiocarbamates and Thiuram Disulphide on Apple, Lettuce, Potato, Strawberry and Tomato Matrix

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Abstract

The control of pesticide residues in agricultural products and food is important for the assurance of healthy food. During the period from 2001 to 2005 when the Agricultural Institute of Slovenia used to carry out the official surveillance of pesticide residues in fruit, vegetable and cereals, dithiocarbamates were most frequently found. This article describes the validation of the method which has been used for the determination since its introduction in 1999. The method is linear from 0.04 mg/kg to 5.0 mg/kg, the limit of detection is 0.004 mg/kg and the limit of quantitative determination is 0.013 mg/kg.

Key words: pesticides, dithiocarbamates, maneb group, GC-MS.

Introduction

The Agricultural Institute of Slovenia analysed agricultural products from 1987 for the pesticide residue content trough specialised projects. From 1999 the control was carried out through national monitoring. In 2004 the national monitoring of pesticide residues was suspended due to a change in legislation. The monitoring of pesticide residues on the market currently falls under the supervision of the Ministry of Health whereas the Ministry of Agriculture, Forestry and Food (MAFF) is responsible for the monitoring of pesticide residues in agricultural products prior to it being put on the market. MAFF has appointed the Agricultural Institute of Slovenia to perform the official analyses.

Residues of active substances, which were most frequently found in the agricultural products of the Slovene market producers from 2001 to 2005, belonged to the dithiocarbamate group: maneb, mancozeb, metiram, propineb and zineb. According to their use dithiocarbamates belong to the group of fungicides. It is important to have a reliable method which enables the quick and simple detection of this group for official monitoring.

Laboratories which routinely determine dithiocarbamates in fruit and vegetable generally use the Keppel method [1-4]. According to this method [5-7] dithiocarbamates and thiuram disulphides are heated with a solution of stannous (II) chloride and hydrochloric acid yielding carbon disulphide. This is further distilled and passed through two absorption tubes: the first one contains a solution of lead acetate and the second a solution of sodium hydroxide. Carbon disulphide is then collected in the ethanol solution of cupric (II) acetate and diethanolamine. Two yellow cupric-N,N-bis(2-hydroxyethyl) dithiocarbamate complexes are formed and measured jointly by spectrophotometry. The method has been improved [8-9] by determining the carbon disulphide formed during the heating of dithiocarbamates and thiuram disulphides with the solution of stannous (II) chloride and hydrochloric acid in the head-space by gas chromatography with electron-capture detection or with flame-photometric detection in the sulphur-mode. Since 1999 the Agricultural Institute of Slovenia has been using a method where fruit, vegetable and cereal samples are heated in a two-phase system of isooctane / stannous (II) chloride in diluted hydrochloric acid. The carbon disulphide which is formed is dissolved in the organic phase (isooctane) and determined by gas chromatography with mass selective detector. This article presents the validation of this method in five matrixes: apple, lettuce, potato, strawberry and tomato.

Experimental

1. Materials

Solutions of carbon disulphide:

A stock standard solution of carbon disulphide (Sigma) in isooctane (p.a. grade) with approximate concentration 630 μg CS_2/mLwas prepared as follows:

50 μL of carbon disulphide were added to a 100 mLvolumetric flask containing approximately 95 mLof isooctane. Because of its volatility, carbon disulphide was added using a syringe under the surface of the solvent. The flask was weighed before and after the addition of carbon disulphide and then filled to the designated mark with isooctane. At the end the actual concentration of the solution was calculated.

An intermediate solution with the concentration of approximately 6.3 μg CS_2/ml, which is equivalent to 5.0 mg CS_2/kg of sample, was prepared from this solution. Working solutions with the concentrations: 0.05, 0.06, 0.25, 0.63, 1.26 and 3.15 μg CS_2/mLwere prepared from the intermediate solution, which is equivalent to 0.04, 0.05, 0.20, 0.50, 1.01 and 2.52 mg CS_2/kg of the sample. Because of the volatility of carbon disulphide and isooctane, laboratory glassware needs to be quickly and tightly closed.

Solutions are used to calibrate a gas chromatograph with mass selective detector. The standards for calibration are stable for one week when kept in vials in a refrigerator.

Solutions of thiram:

Stock standard solutions of thiram (Dr. Ehrenstorfer) were prepared in acetone (p.a. grade) Working solutions with different concentrations were prepared from the stock standard solutions.

The solutions may be used for one month when refrigerated.

Solution of stannous (II) chloride:

20 g stannous (II) chloride dihydrate were dissolved in 500 mLconcentrated hydrochloric acid (35%-37%); the solution was decanted in a one-litre volumetric flask and filled to the designated mark with deionised water (Milli Q). The solution has to be prepared prior to use.

2. Procedure

50 g of the sample was placed in a laboratory bottle where 40 mLisooctane was added with a pipette and a 100 mLsolution of stannous (II) chloride was added using a graduated cylinder. The closed bottle was placed in a water bath at 80 ºC and heated for 60 minutes. The solution was mixed approximately every 15 minutes. The bottle was taken out of the bath after one hour, cooled to room temperature and the upper phase was transferred into a GC vial using a Pasteur pipette.

Spiked samples were treated in the same way except that 1 mLsolution of thiram with concentration 50 μg/mLwas added to 50 g of the sample, which did not contain dithiocarbamates or thiuram disulphides, for the preparation of the fortification level 1 mg thiram/kg sample. Two moles of carbon disulphide were formed from one mole of thiram (M = 240.4 g/mol). Spiked samples of other fortification levels were prepared.

For each series of analyses a reagents blank was prepared.

3. Determination

Chromatographic conditions of GC-MS system:

Liner	HP 5183-4647
Injector temperature	250 ºC
Injection type	Pulsed Splitless
Precolumn	2 m * 0.25 mm
Column	HP 5 MS, 30 m * 0.25 mm; film 0.25 μm
Temperature gradient	50 ºC; 2.2 min 35 ºC/min; from 50 ºC to 270 ºC 270 ºC; 2 min
Ion source temperature	230 ºC
Auxiliary temperature	280 ºC
Detector temperature	150 ºC
Carrier gas	Helium 6.0; constant flow 1.0 mL/min
Injection volume	1 μL
Detection	Selective Ion Monitoring; target ion 76 m/z

Results and discussion

1. Linearity, limit of detection, limit of quantification

Linearity was verified by using the solutions of carbon disulphide in isooctane (five repetitions for one concentration level, seven concentration levels for the calibration curve). Linearity and range were determined by linear regression, using the F test. Linear model is fit and remains linear over the range from 0.04 mg/kg to 5.0 mg/kg; R^2 is 1.000. Limit of detection 0.004 mg/kg (LOD = 3xS/N) and limit of quantification 0.013 mg/kg (LOQ = 10xS/N) were calculated from S/N ratio. According to the MRLs for dithiocarbamates in potato (MRL = 0.1 mg CS_2/kg sample[10]), lettuce (MRL = 5.0 mg CS_2/kg sample[10]), apple (MRL = 3.0 mg CS_2/kg sample[10]), tomato (MRL = 3.0 mg CS_2/kg sample[10]) and strawberry (MRL = 2.0 mg CS_2/kg sample[10]), the calculated values for limit of detection and limit of quantification are both appropriate and thus the method is fit for purpose.

2. Trueness

Trueness was verified by checking the recoveries. Ten spiked samples were prepared and injected twice respectively. The average of recoveries was calculated. The results are given in Table 1.

Table 1. Recoveries for apple, lettuce, potato, strawberry and tomato

Matrix	Recovery (%)	RSD (%)	Level (mg CS_2/kg sample)
Apple	96.0	1.6	3.0
Lettuce	99.3	2.0	5.0
Potato	111.1	1.8	0.1
Strawberry	99.6	0.9	2.0
Tomato	100.9	1.2	3.0

Since 2003 we have been participating in a proficiency testing scheme organised by BIPEA (Bureau interprofessionnel d´études analytiques). Table 2 shows the results for the determination of dithiocarbamates content in lettuce obtained from the scheme in the period from 2003 to 2005.

3. Precision

For the determination of precision[11], i.e. repeatability and reproducibility, spiked samples of apple, lettuce, potato, strawberries and tomato were analysed. Within the period of 10 days two spiked samples were prepared each day. Both were injected twice. Then standard deviation of repeatability of the level and standard deviation of reproducibility of the level were both calculated. The results are given in Table 3.

4. Uncertainty of repeatability and uncertainty of reproducibility

Uncertainty of repeatability and uncertainty of reproducibility were calculated by multiplying standard deviation of repeatability and standard deviation of reproducibility by Student's *t* factor for 9 degrees of freedom and 95% confidence level ($t_{95;9} = 2.262$).

$$U_r = t_{95;\,9} \text{ x } s_r \ ; \ U_R = t_{95;\,9} \text{ x } s_R$$

The results are presented in Table 4.

Conclusions

According to the validation, the method is suitable for the determination of dithiocarbamates and thiuram disulphides residue content in apple, lettuce, potato, strawberry and tomato. The system is linear in the range from 0.04 mg/kg to 5.0 mg/kg. The limit of

Table 2. Dithiocarbamates content (mg/kg) in lettuce

Circuit-19: *Pesticides*	Reference	Tolerance	Maximum	Minimum	Our result
January 2003	0.140	0.070	0.210	0.070	0.114
March 2003	1.192	0.596	1.788	0.596	1.183
April 2003	12.700	6.350	19.050	6.350	8.852
October 2003	6.278	3.139	9.417	3.139	4.609
February 2004	0.287	0.144	0.431	0.143	0.262
April 2004	0.333	0.166	0.500	0.166	0.269
October 2004	0.603	0.241	0.844	0.362	0.651
December 2004	0.364	0.146	0.510	0.218	0.283
February 2005	0.503	0.201	0.704	0.302	0.467
April 2005	4.216	1.686	5.902	2.530	3.826
October 2005	5.752	2.301	8.053	3.451	5.909

Table 3. Standard deviation of repeatability and reproducibility of the method, in mg/kg

	Apple	Lettuce	Potato	Strawberry	Tomato
Spiking level (mg CS_2/kg)	3.00	5.0	0.100	2.00	3.00
Means of the levels (mg CS_2/kg)	2.85	4.8	0.101	1.93	2.88
Standard deviation of repeatability (s_r)	0.05	0.1	0.001	0.01	0.03
Standard deviation of reproducibility (s_R)	0.11	0.1	0.008	0.08	0.10

Table 4. Uncertainty of repeatability and reproducibility of the method, in mg/kg

	Apple	Lettuce	Potato	Strawberry	Tomato
Spiking level (mg CS_2/kg)	3.0	5.0	0.100	2.00	3.0
Uncertainty of repeatability (U_r)	0.1	0.2	0.002	0.03	0.1
Uncertainty of reproducibility (U_R)	0.2	0.3	0.017	0.19	0.2

detection (0.004 mg/kg) and the limit of quantitative determination (0.013 mg/kg) ensure that the method is suitable for the determination of residues regarding the prescribed MRLs. Inter-laboratory comparisons and recoveries have proved that the method is precise and accurate. The validation was performed in five matrixes and the method may be extrapolated to fruit, vegetables and cereals.

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Povzetek

Kontrola ostankov pesticidov v kmetijskih pridelkih in hrani je pomembna za zagotavljanje zdrave hrane. V obdobju od leta 2001 do 2005, ko smo na Kmetijskem inštitutu Slovenije izvajali uradni nadzor ostankov pesticidov v sadju, zelenjavi in žitih, smo najpogosteje našli ostanke ditiokarbamatov. V tem članku opisujemo validacijo metode, ki smo jo uporabljali za določevanje od njene vpeljave leta 1999. Metoda je linearna od 0,04 mg/kg do 5,0 mg/kg, meja detekcije je 0,004 mg/kg in meja kvantitativne določitve 0,013 mg/kg.