

Emese GÁL¹, Łukasz DĄBROWSKI²

¹Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, Hungarian Line of study, Arany János str, Kolozsvár, 400028, Romania.

²UTP University of Science and Technology, Department of Food Analysis and Environmental Protection, Faculty of Chemical Technology and Engineering, Seminaryjna Street, 85-326 Bydgoszcz, Poland
emese.gal@ubbcluj.ro

Paper and cardboard is the most common packaging material being utilized as primary and secondary packaging materials, often in forms adapted to direct contact with foodstuffs. Pyrimethanil (Pyr), imazalil (Ima), thiabendazole (Tbz) and orto-phenyl phenol (OPP) are commonly applied in citrus packhouses for the control of green mould, caused by *Penicillium digitatum*. The chosen compounds are commonly detected in fruit, vegetable and their packaging. The present study presents a method for the non-thermal Focused UltraSound Extraction (FUSE) technique and a GC/MS detection method for the fungicides listed above. The developed method showed a good linearity ($R^2 > 0.99$) and precision, yielding relative standard deviations (RSD) of less than 15% for reproducibility and 18% for repeatability.

Table 1. GC/MS method validation parameters (calibration in 5 point, three replicate injection)

Std. name	IS conc. µg/ml	LOD (µg/ml, S/N 3)	LOQ (µg/ml, S/N 10)	RT (min)	R ²	Accuracy (%)	Quantitation ions m/z
OPP	-	0.05	2	7.014	0.9965	90-102	170, 169, 141
Pyr	-	0.05	2	8.043	0.9933	84-107	198, 199, 200
TBZ	-	1.25	2	9.363	0.9982	92-104	174, 175, 201
Ima	-	0.1	2	9.689	0.9976	95-107	173, 215, 217
HCB-IS	4	-	-	7.792	-	-	282, 284, 286
Lin-IS	2	-	-	7.995	-	-	218, 219, 221
Tpp-IS	2	-	-	10.709	-	-	201, 215, 325, 326

HCB-hexachlorbenzene, Lin-lindane, Tpp-triphenylphosphine

Table 2. Characteristics of the selected cardboard boxes.

Cardboard box (cardboard number/ used pesticides)	Utilization (fruit)	Originate
Cb002-Ima, Tbz, Pyr	oranges	South Africa
Cb005-Ima Tbz, Pyr	oranges	Spain
Cb006-Ima, Pyr	lemon	Turkey
Cb009-Ima	mandarin	Chile
Cb010-Ima, Tbz, Pyr	oranges	South Africa
Cb015-Ima	mandarin	Spain
Cb016-Ima, Tbz, Pyr	lemon	Turkey
Cb017-paper from lemon packages Ima, Tbz, Pyr	lemon	Turkey
Cb019-bio cardboard no preservatives	lemon	Spain
Cb020-bio cardboard no preservatives	Lemon and leaf	Spain

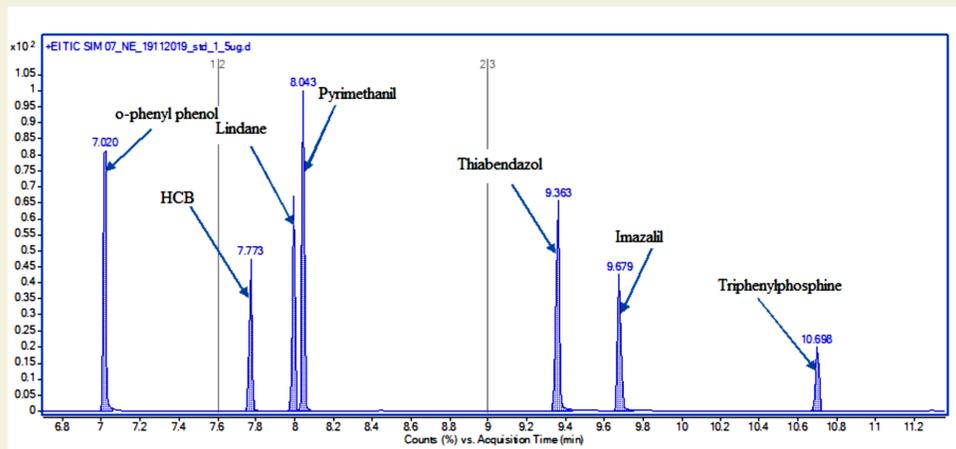


Fig.1. GC/MS chromatogram of IS and STD used.

Table 3. FUSE method development parameters.

FUSE method	Sample	Solvent	Time/ quantity of solvent	Calculated recovery (%)			
				OPP	PYR	TBZ	IMA
100 % power, 0,5 cycle	5 x 5 cm (~0,6 g), one piece	ACN	1,5 min, 8ml	47	63	8.34	67.9
		MeOH	1,5 min, 8ml	83	83	28.7	103.6
		Acetone	1,5 min, 8ml	4.62	16	20.3	19.3
		DCM: MeOH/ 92:8 (v/v)	1,5 min, 8ml	59.4	74	62.9	80.1
		DCM: MeOH/ 92:8 (v/v)	1,5 min x 2, 16 ml	23.3	34.4	16.9	34.7
		DCM: MeOH/ 92:8 (v/v)	3 min, 8 ml	29	36	21	34.4
		DCM: MeOH/ 92:8 (v/v)	3 min x 2, 16 ml	30	35	23.6	29.3
		DCM: MeOH/ 92:8 (v/v)	4,5 min, 8 ml	28	34.5	19.3	44.4
		DCM: MeOH/ 92:8 (v/v)	1,5 min x 3, 24 ml	22	33.5	22.8	36.1
		DCM: MeOH/ 92:8 (v/v)	5 min, 8 ml	22	33.4	16.8	37.9
		DCM: MeOH:ACN/ 87:6.6:6.6 (v/v)	3 min, 8 ml	50	70	57.5	71.4
		DCM: MeOH:ACN/ 87:6.6:6.6 (v/v)	1,5 min x 2, 16 ml	33	45	22.1	58.3
		DCM: MeOH:ACN/ 76:12:12 (v/v)	3 min, 8 ml	92	89.3	92	89.9
		DCM: MeOH:ACN/ 76:12:12 (v/v)	1,5 min x 2, 16 ml	61	81.4	84.9	89.4
100 % power, 0,8 cycle	5 x 5 cm (~0,6 g), one piece	DCM: MeOH/ 92:8 (v/v)	1,5 min x 2, 16 ml	72.1	83.5	19.9	91.3
		DCM: MeOH/ 92:8 (v/v)	3 min, 8 ml	11	18.12	> LOQ	17.1

Table 4. Fungicide quantities found in cardboards.

Cb. sample	Pyr (µg/ml)	Tbz (µg/ml)	Ima (µg/ml)
Cb002	15.6366	2.7667	5.5843
Cb005	2.5131	1.1433	3.7215
Cb006	2.7032	-	0.3307
Cb009	3.6507	-	0.6855
Cb010	-	4.7204	2.7992
Cb012	-	12.5828	5.7469
Cb016	6.1443	-	0.3577
Cb017	-	-	0.3479

Equipments

- > Agilent 7890B GC system and a 5977B MSD (Agilent Technologies)
- > Hielscher UP 100H sonicator/homogenizer provided with a 3-mm cylindrical titanium alloy probe



Fig.2. Cardboard boxes inscriptions with used fungicide.

Extraction procedure: 5 x 5 cm cardboard sample was weighed (aprox. 0.6 g) in a 9 mL vial. 8 ml solvent mixture (DCM: ACN: MeOH/ 76: 12: 12) were added and the sample were ultrasonicated 3 min. at 100% power 0.5 s cycle using micro-tip. After extraction the solvent were poured off from the cardboard. To the obtained extract solution was added 100 µl 1-octanol as solvent keeper. The extracts were evaporated under nitrogen stream at 40 °C. After evaporation the obtained mixture were transferred to a micro vial and internal standards were added.

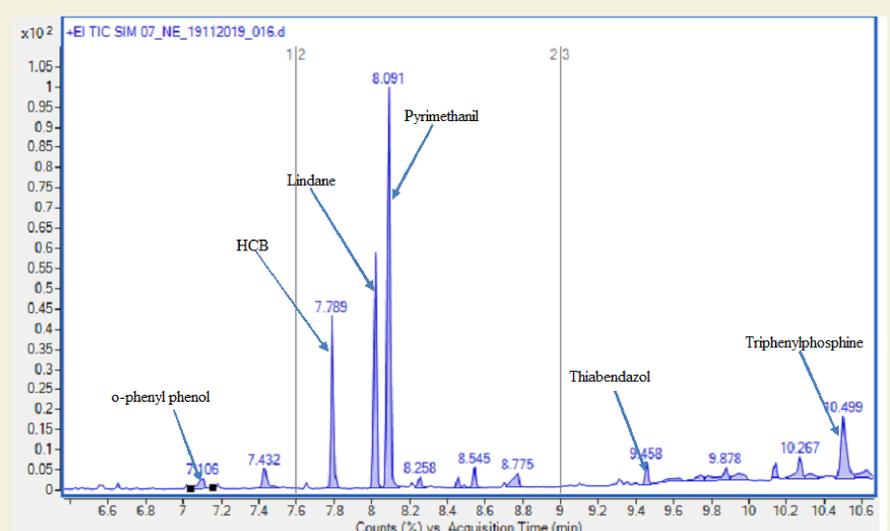


Fig.3. GC/MS chromatogram of a real sample Cb002.

Conclusion: A procedure for analyzing five chemical contaminants in cardboard, used in citrus fruits packaging, was developed. For extraction a new FUSE method was developed and optimized using a solvent mixture to obtain a high recovery for fungicide. GC/MS analysis was developed using SIM method. Applicability of the method was illustrated using commercial cardboard boxes. The results suggest that the presence of used fungicides can be detected even after a long period after the treatment.

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