Combined Thin-Layer Chromatography and Mass Spectrometry for the Screening of Pesticides in Samples derived from Biological Origins

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Abstract

This study describes a method for off-line coupling of thin-layer chromatography (TLC) with electron impact ionization–mass spectrometry for routine determination of pesticides in Toxicology and forensic medicine. Six TLC solvent systems are described for 151 different pesticides, and 8-peak mass-spectra generated from full EI mass-spectra are listed.

Introduction

The combination of thin-layer chromatography (TLC) and mass spectrometry (MS) is commonly used because of its different benefits. Even more important than the aspect of moderate cost is the fact that TLC plates can act as a storage system if the separation of substances and their characterization via MS has to be performed at different locations, which is quite often the case in forensic medicine. This high degree of flexibility is not offered by any other method concerning coupled systems (e.g., GC–MS).

The screening of biological samples in forensic chemistry or toxicology is performed in two main areas: the first is the detection of known compounds and the second is the detection of unknown compounds.

The detection of known compounds is a rather routine procedure. It can be done by proven methods and by the use of the corresponding compilation of analytical data for each technique (1–4).

The identification of unknown substances is somewhat difficult, but the more interesting part of toxicological research, and the identification, can be successfully achieved by TLC–MS (5).

The role of TLC as a simple low cost method is still of importance. Furthermore this method enables the simultaneous handling of several samples. TLC negative samples do not need further analysis and, in consequence, the number of analytical procedures can be dramatically reduced. In this way, subsequent spectroscopy is only required for positive fractions.

GC–MS is normally used as a confirmation technique (6). This often requires complex sample preparations like extraction and derivatization, which are followed by time consuming GC runs. This study describes the usefulness of off-line coupled TLC–MS as a confirmation technique for direct insertion probe EI–MS of pesticides after TLC separation.

There are other ionization methods (such as FAB–MS) described and there are also methods combined with TLC that have been reviewed by Wilson (7) that are especially useful for the detection of thermally labile compounds or for compounds with high polarity.

Experimental

Off-line TLC-EI–MS

Silica P_{254} (Merck, Darmstadt, Germany) TLC plates (20 cm x 10 cm) were used for hR_f determination and for all analyses of biological species. The composition (v/v) of the mobile phase were: (S1) methanol–25% aqueous ammonia, 100:1.5 (v/v); (S2) cyclohexane–toluene–diethylamine, 75:15:10 (v/v/v); (S3) chloroform–methanol, 90:10 (v/v); (S4) *n*-hexane–acetone, 80:20 (v/v); (S5) toluene–acetone, 95:5 (v/v); and (S6) chloroform–acetone, 50:50 (v/v).

The results achieved with these mobile phases were similar to those described elsewhere (8). The corrected hRf x 100 values (8) of 151 pesticides are listed in Table I.

The reagents used for color reaction were Dragendorff (modified), Ludy Tenger, potassium iodoplatinate, palladium chloride, ferric chloride-sulfuric acid, mercury nitrate-mercury sulfate as described in [2].

EI mass spectra were obtained by a Finnigan MAT 212 (Bremen, Germany) instrument equipped with a spectrosystem SS 300. The compounds were transferred into quartz crucibles to the direct inlet system of the instrument and evaporated by TIC-con-

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Table I. Corrected hRf x 100 Values of 151 Pesticides

No	Eight-peak mass spectrum								$M^{+\cdot}$	hRF in mobile phase			compound name	Elemental composition
	a	b	c	d	e	f	g	h		1	2	3		
1	43	86	234	236	128	110	70	152	269	85	88	87	Diallat	C10H17NOCl2S
2	43	86	268	128	270	143	145	147	303	69	89	90	Triallat	C10H16NOCl3S
3	44	201	186	173	158	138	68	96	204	73	04	68	Simazin	C7H15N5Cl
4	45	160	188	237	146	224	269	132	269	89	89	95	Alachlor	C14H20NO2Cl
5	53	127	223	164	171	153	144	99	223	87	44	90	Chlorbufam	C11H10NO2Cl
6	58	41	86	144	100	76	100	69	190	85	26	88	Aldicarb	C7H14N2O2S
7	58	105	42	88	45	47	162	71	162	78	06	74	Methomyl	C5H10N2O2S
8	61	46	248	250	160	162	219	221	248	85	15	90	Linuron	C9H10N2O2Cl2
9	61	46	126	214	99	128	153	216	214	82	27	86	Monolinuron	C9H11N2O2Cl
10	72	245	247	290	292	202	217	182	290	86	07	80	Chloroxuron	C15H15N2O2Cl
11	72	44	212	214	77	104	132	140	212	84	07	93	Chlortoluron	C10H13N2OCl
12	72	232	234	44	57	83	97	187	232	82	00	69	Diuron	C9H10N2OCl2
13	72	228	183	168	45	230	185	140	228	80	03	69	Metoxuron	C10H13N2O2Cl
14	77	220	221	105	88	222	223	51	221	77	00	57	Pyrazon	C10H8N3OCl
15	79	107	183	149	264	271	313	349	347	79	00	60	Captafol	C10H9Cl4NO2S
16	79	149	107	117	119	264	299	236	299	79	00	85	Captan	C9H8NO2Cl3S
17	84	133	162	42	161	51	55	119	162	54	39	35	Nicotin	C10H14N2
18	87	93	58	125	229	79	143	197	197	77	06	64	Dimethoat	C5H12NO3PS2
19	88	109	142	125	60	79	112	230	230	83	00	93	Demethon-S-methyl	C6H15O3PS2
20	88	274	186	276	60	61	97	125	274	89	93	93	Disulfoton	C8H19O2PS3
21	97	197	199	314	258	286	349	125	349	87	96	80	Chlorpyrifos	C9H11NO3Cl3PS
22	109	169	125	76	47	45	59	105	246	70	00	73	Demethon-S-methylsulfoxid	C6H15O4PS2
23	109	149	99	81	139	247	275	219	275	77	22	85	Paraoxon	C10H14NO6P
24	109	125	263	79	47	6 3	93	200	263	82	40	85	Parathionmethyl	C8H10NO5PS
25	109	185	145	220	79	187	222	147	220	80	19	82	Dichlorvos	C4H7O4Cl2P
26	110	152	43	58	64	81	137	153	209	86	27	88	Propoxur	C11H15NO3
27	112	197	58	182	43	44	155	69	197	96	00	12	Hydroxiatrazin	C8H15N5O
28	123	77	167	224	226	44	91	332	332	86	20	93	Dichlofluanid	C9H11N2O2Cl2S2F
29	125	127	165	167	196	197	137	139	232	89	79	93	Chlorphenpropmethyl	C10H10O2Cl2
30	125	287	285	79	109	93	97	167	320	96	75	93	Fenchlorphos	C8H8O3Cl3PS
31	127	67	193	237	44	109	72	111	237	76	11	66	Dicrotophos	C8H16O5NP
32	127	192	109	224	43	67	164	95	224	80	06	85	Mevinphos	C7H13O6P
33	132	77	160	104	97	65	129	51	345	80	16	85	Azinphos-ethyl	C12H16N3O3PS2
34	137	227	155	121	109	77	94	157	344	85	80	75	Methoxychlor	C16H15O2Cl3
35	139	141	251	253	111	113	141	75	368	89	80	91	Dicofol	C14H9OCl5
36	142	157	200	114	144	159	202	116	200	76	00	33	Metoxuron-didesmethyl	C8H9N2O2Cl
37	142	157	214	114	144	159	216	116	214	79	00	51	Metoxuron-monodesmethyl	C9H11N2O2Cl
38	142	107	77	214	169	89	144	216	214	96	00	13	Mecoprop	C10H11O3Cl
39	143	87	43	235	115	77	132	218	235	85	38	90	Carboxin	C12H13NO2S
40	144	115	116	201	127	136	89	71	201	58	06	68	Carbaryl	C12H11O2N
41	151	126	166	223	51	58	108	79	223	86	14	88	Bendiocarb	C11H13NO4
42	153	43	53	69	81	82	110	136	234	82	10	81	Lenacil	C13H18N2O2
43	156	93	281	125	43	55	63	68	281	78	02	32	Menazon	C6H12N5O2PS2
44	156	110	58	79	126	47	95	141	213	65	10	52	Omethoat	C5H12NO4PS
45	159	191	43	105	88	132	146	119	290	76	03	92	Benomyl	C14H18N4O3
46	160	135	44	294	77	92	104	251	294	89	06	72	Triamiphos	C12H19N6OP
47	160	132	77	93	104	125	147	172	317	80	15	85	Azinphos-methyl	C10H12N3O3PS2
48	164	136	135	221	96	163	108	69	221	83	10	87	Methabenzthiazuron	C10H11N3OS
49	169	109	125	142	79	47	127	93	262	74	00	76	Demethon-S-methylsulfon	C6H15O5PS2
50	170	134	199	243	172	198	245	108	243	80	00	06	Benazolin	C9H6O3NClS
51	171	173	100	75	136	50	61	109	171	96	80	92	Dichlobenil	C7H3NCl2
52	173	175	220	222	203	191	97	109	220	90	00	06	Dicamba	C8H6O3Cl2
53	173	174	146	230	105	57	77	89	230	89	00	85	Pivalylindandion	C14H14O3
54	185	226	241	170	157	43	68	71	241	77	06	70	Terbutyne	C10H19N5S
55	191	159	105	119	146	132	78	90	191	82	07	60	Carbendazim	C9H9N3O2
56	196	198	268	270	200	223	225	272	268	85	00	09	Fenoprop	C9H7O3Cl3

Table I (continued). Corrected hRf x 100 Values of 151 Pesticides

No	Eight-peak mass spectrum								$M^{+\cdot}$	hRF in mobile phase			compound name	Elemental composition
	a	b	c	d	e	f	g	h		1	2	3		
57	198	42	57	61	104	145	214	183	214	85	38	87	Metribuzin	C8H14N4OS
58	200	215	58	173	68	122	132	138	215	77	04	70	Atrazin	C8H14N5Cl
59	205	207	231	233	188	190	260	262	260	80	00	80	Bromacil	C9H13N2O2Br
60	211	163	147	117	240	77	65	89	240	92	00	91	Dinoseb	C10H12N2O5
61	213	198	171	58	57	82	99	124	213	73	04	68	Desmetryne	C8H15N5S
62	214	229	172	187	58	99	43	152	229	75	05	72	Propazine	C9H16N5Cl
63	221	97	232	373	237	265	65	91	373	87	67	93	Pyrazophos	C14H20N3O5PS
64	227	212	58	170	185	68	43	110	227	76	08	71	Ametryne	C9H17N5S
65	241	184	226	58	199	106	43	68	241	76	05	74	Prometryne	C10H19N5S
66	266	264	268	229	231	168	109	124	264	88	83	93	Chlorthalonil	C8N2Cl4
67	266	264	268	165	167	202	200	230	264	88	00	57	Pentochlorphenol	C6HOCl5
68	267	269	323	325	81	295	297	109	358	88	53	93	Chlorfenvinphos	C12H14O4Cl3P
69	278	125	109	153	168	169	79	93	278	85	79	95	Fenthion	C10H15O3PS2
70	279	281	97	223	251	162	314	109	314	89	93	94	Dichlofenthion	C10H13O3Cl2PS
71	291	109	97	137	155	139	235	263	291	82	60	85	Parathionethyl	C10H14NO5PS
72	292	188	121	130	115	91	65	77	292	73	13	74	Coumatetralyl	C19H16O3
73	295	249	214	237	142	265	179	109	293	88	92	94	Quintozene	C6O2NCl5
74	299	301	342	344	285	187	121	132	342	87	00	84	Cumachlor	C19H15O4Cl
75	306	264	335	43	248	290	316	318	335	85	80	88	Trifluralin	C13H16N3O4F3
76	316	274	300	345	258	43	328	216	345	85	24	90	Nitralin	C13H19N3O6S
77	322	202	97	266	238	294	65	121	322	96	86	93	Sulfotep	C8H20O5P2S2
78	331	329	333	125	109	314	316	213	364	81	80	87	Bromophos-methyl	C8H8O3Cl2BrPS
79	359	357	361	303	331	97	242	213	392	81	80	87	Bromophos-ethyl	C10H12O3Cl2BrPS
80	43	58	68	255	113	152	184	240	255	35	35	95	Dipropetryn	C11H21N5S
81	43	128	86	189	132	41	89	75	189	69	63	97	EPTC	C9H19NOS
82	43	161	207	79	137	133	105	286	286	25	50	95	Ethofumesate	C13H18O5S
83	43	93	179	137	120	65	41	77	179	39	57	94	Propham	C10H13NO2
84	51	121	53	52	50	77	105	198	198	06	38	45	DNOC	C7H6N2O5
85	53	110	236	68	127	164	179	207	236	20	29	93	Buturon	C12H13ClN2O
86	57	127	41	43	55	88	243	42	371	04	19	39	Ioxynil	C7H3J2NO
87	57	231	103	125	153	186	203	41	288	63	90	99	Terbufos	C9H21O2PS3
88	57	208	85	181	110	128	293	236	293	22	23	94	Triadimefon	C14H16ClN3O2
89	61	46	294	206	292	60	45	63	292	22	30	92	Chlorbromuron	C9H10ClBrN2O2
90	66	263	293	79	91	101	329	364	362	89	98	99	Aldrin	C12H8Cl6
91	72	89	127	198	45	99	141	154	198	13	05	79	Cycluron	C11H22N2O
92	72	241	286	77	90	92	226	63	286	08	05	83	Difenoxuron	C16H18N2O3
93	72	164	44	65	42	77	91	51	164	11	07	81	Fenuron	C9H12N2O
94	72	232	44	213	187	145	113	95	232	16	09	87	Fluometuron	C10H11F3N2O
95	72	198	44	200	73	42	99	100	198	13	07	83	Monuron	C9H11ON2Cl
96	72	166	238	167	42	44	138	109	238	26	17	92	Pirimicarb	C11H18N4O2
97	77	97	129	157	125	141	103	298	298	42	86	97	Phoxim	C12H15N2O3PS
98	79	108	263	345	380	237	277	279	378	65	87	99	Dieldrin	C12H8OCl6
99	79	109	110	139	145	80	112	95	256	04	02	62	Trichlorfon	C4H8Cl3O4P
100	81	263	281	279	261	265	79	82	378	71	90	99	Endrin	C12H8OCl6
101	84	57	43	42	85	69	54	41	84	00	00	06	Amitrol	C2H4N4
102	88	62	61	53	277	63	89	87	275	8	16	40	Bromoxynil	C7H3Br2NO
103	88	44	120	240	77	55	56	76	240	19	51	94	Thiram	C6H12N2S4
104	94	95	141	64	47	46	79	80	141	01	00	37	Methamidophos	C2H8NO2PS
105	97	121	65	47	154	93	125	234	234	64	91	99	Chlormephos	C5H12ClO2PS2
106	109	125	79	297	128	47	63	93	297	31	71	97	Chlorthion	C8H9ClNO5PS
107	109	137	246	110	81	63	65	77	246	59	89	97	Fonofos	C10H15OPS2
108	109	137	246	290	110	305	276	69	305	49	75	100	Pirimiphos-methyl	C11H20N3O3PS
109	115	57	83	41	172	143	161	218	218	20	17	94	Thiofanox	C9H18N2O2S
110	123	136	107	79	93	91	81	67	302	48	52	97	Bioallethrin	C19H26O3
111	125	173	127	93	99	158	143	285	330	31	53	96	Malathion	C10H19O6PS2
112	125	93	109	79	46	203	63	171	466	23	75	98	Temephos	C16H20O6P2S3

Table I (continued). Corrected hRf x 100 Values of 151 Pesticides

No	Eight-peak mass spectrum								$M^{+\bullet}$	hRF in mobile phase			compound name	Elemental composition
	a	b	c	d	e	f	g	h		1	2	3		
113	126	72	264	138	109	67	193	70	299	07	02	79	Phosphamidon	C10H19ClNO5P
114	127	109	125	277	260	79	192	93	277	32	76	95	Fenitrothion	C9H12NO5PS
115	127	192	109	67	224	164	43	79	224	10	07	99	Mevinphos	C7H13O6P
116	127	67	97	58	109	79	192	223	223	01	01	12	Monocrotophos	C7H14NO5P
117	128	141	152	215	231	168	77	63	268	12	16	90	Dichlorphen	C13H10Cl2O2
118	136	94	43	95	96	79	47	125	183	00	00	42	Acephate	C4H10NO3PS
119	137	238	181	240	44	138	92	65	346	33	67	97	Tolylfluanid	C10H13Cl2FN2O2S2
120	141	200	77	143	155	125	142	202	200	00	00	03	MCPA	C9H9ClO3
121	142	106	144	87	43	77	45	108	228	08	06	59	MCPB	C11H13ClO3
122	145	85	93	125	146	69	58	147	302	29	56	95	Methidathion	C6H11N2O4PS3
123	153	141	63	113	127	90	125	310	310	18	24	93	Diflubenzuron	C14H9ClF2N2O2
124	158	43	97	139	126	41	93	74	242	33	28	96	Ethoprophos	C8H19O2PS2
125	161	77	97	91	65	51	172	177	313	21	38	94	Triazophos	C12H16N3O3PS
126	162	164	220	161	63	133	111	222	436	00	02	07	2,4-D	C8H6O3Cl2
127	162	164	234	189	45	63	98	126	234	03	02	09	Dichlorprop	C9H8Cl2O3
128	164	149	122	57	123	221	131	91	221	17	20	92	Carbofuran	C12H15NO3
129	167	133	135	104	132	122	105	78	300	11	17	93	Phenmedipham	C16H16N2O4
130	173	374	165	201	89	356	77	105	374	00	01	45	Chlorophacinon	C23H15ClO3
131	173	174	146	89	105	230	77	57	230	17	45	80	Pindone	C14H14O3
132	173	175	255	145	147	109	75	240	255	33	52	95	Propyzamide	C12H11Cl2NO
133	174	201	129	63	64	90	175	202	201	07	03	67	Thiabendazole	C10H7N3S
134	179	137	152	304	248	276	227	199	304	47	50	96	Diazinon	C12H21N2O3PS
135	182	121	184	154	367	97	241	58	367	31	67	97	Phosalone	C12H15ClNO4PS2
136	184	155	156	92	129	102	77	63	184	09	08	80	Fuberidazole	C11H8N2O
137	195	197	241	207	237	239	243	265	404	77	95	98	Endosulfan	C9H6O3Cl6S
138	196	198	161	200	163	86	242	245	240	00	00	00	Pichloram	C6H3Cl3N2O2
139	200	186	214	229	68	72	43	96	229	43	42	95	Trietazine	C9H16ClN5
140	203	55	201	303	83	301	199	41	432	19	02	87	Triforine	C10H14Cl6N4O2
141	211	163	147	117	240	77	89	65	282	40	81	98	Dinoseb-acetat	C12H14N2O6
142	219	181	183	109	111	254	290	85	288	51	92	98	Lindane	C6H6Cl6
143	221	163	164	44	149	122	42	147	221	01	01	19	Formetanate	C11H15N3O2
144	225	67	44	173	240	198	171	145	240	16	12	90	Cyanazine	C9H13ClN6
145	234	165	244	166	228	91	239	105	321	58	73	96	Trifenmorph	C23H15NO
146	235	165	237	354	199	246	212	239	352	76	98	99	DDT	C14H9Cl5
147	256	58	45	213	43	271	226	212	271	19	11	88	Methoprotryn	C11H21N5OS
148	265	43	121	187	308	145	251	77	308	12	11	91	Warfarin	C19H16O4
149	272	270	56	315	317	85	274	7	385	00	00	03	Cyhexatin	C18H34OSn
150	272	100	337	237	372	65	135	194	370	84	97	99	Heptachlor	C10H5Cl7
151	362	109	226	97	210	364	334	228	362	27	61	96	Coumaphos	C14H16O5PSCl

trolled heating at 200°C. EI spectra were obtained at 100 eV of ionization energy in full-scan mode. The eight peak mass spectra are tabulated in Table I.

Preparation of biological samples

The TLC plates were pretreated with a solution of methanol–1.5% aqueous ammonia (25%), reactivated for 2 h at 150°C in a clean heating oven, and subsequently cooled in a clean closed system .The samples to be determined were obtained from stomach content by liquid–liquid extraction at a neutral pH or by solid-phase extraction (SPE). SPE was performed using 1 mL liquid stomach content diluted with 4 mL of phosphate buffer (pH 6) and vortex mixed for 1 min. The buffered matrix was centrifuged and the supernatant was used for separation on Bond Elute columns (9). The extracts derived from liquid-liquid extraction of fatty samples were further cleaned by liquid-liquid extraction using acetonitrile–petroleum (light fraction) (10).

The extracts were applied to the TLC plate as spots. This method allowed the development of ten extracts simultaneously. If any unknown compounds were detected, the extracts containing these compounds were applied to a pretreated plate as a band and subsequently developed with an appropriate mobile phase. One part of the TLC plate (~ 2 cm) was used for detection of the substances by use of color reactions. One or more bands to be investigated by MS were marked, scratched from the plate, extracted with methanol–dichloromethane (1:1, v/v) under sonication for 5 min, and centrifuged. The supernatant was transferred to another clean tube and evaporated to dryness by means of a gentle stream of nitrogen. The residue was dissolved in methanol (20 mL) and this solution (5 mL) was transferred to a

quartz MS crucible and the solvent was evaporated again. The dry sample was transferred to the MS (5).

Results and Discussion

The hR_f data of 151 pesticides are listed in Table I in addition to eight peak mass spectra under EI. The plot of solvent systems against each other (S1/S2, S1/S3, S2/S3) (Figure 1) confirms the ability of these systems to determine many pesticides. The same results are obtained with solvent systems S4–S6. In addition to EI–MS, the plot of base peak against the hRf shows that with this additional tool a more satisfying identification is achieved (Figure 1, plot EI against S1). The other solvent systems show comparable results.

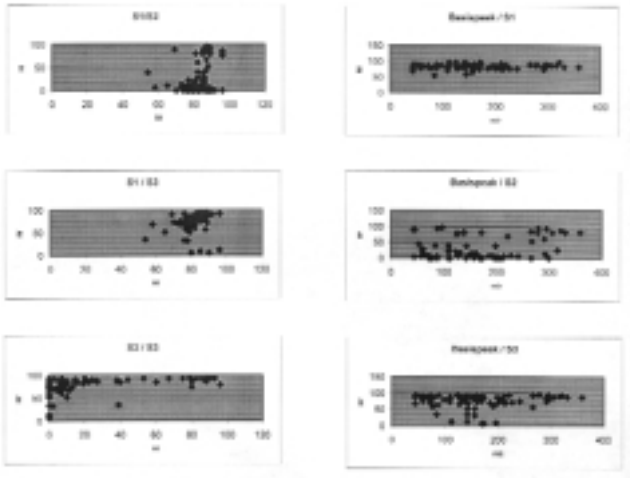

Figure 1. Plot of solvent systems against each other containing S1/S2, S1/S3, S2/S3, Basispeak/S1, Basispeak/S2, and Basispeak S3.

Conclusion

In conclusion, these results show that TLC–MS in any kind of ionization procedure is a valuable method. It can be applied to a very wide range of analytes (5,7) and is comparable to HPLC-based methods. Because of off-line coupling, TLC–MS can even be applied to analytes that are unsuitable for other separation techniques.

This screening technique can be used in practice as an alternative to GC–MS. The most important advantage is the possibility of quick analyses in the case of intoxications in emergency medicine.

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