Analysis of pesticide residues in agricultural products using automated Liner Exchange Difficult Matrix Introduction (LINEX-DMI) technique coupled to Gas Chromatography/ Mass Spectrometry (GC/MS)

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1. Introduction

For the determination of multiple of pesticide resides in foods, the complex preparation is generally required and, therefore, is time-consuming due to the complexity of both the matrices and the interfering compounds. A technique called the LINEX-DMI, in which automatic liner-exchanging (LINEX) is combined with difficult matrix introduction (DMI), has become a powerful tool for overcoming the problems. In the LINEX-DMI approach, the GC liner is automatically exchanged, and the non-volatile matrices such as pigments and lipids trapped in the liner can be removed at each run. With the application of the LINEX-DMI system, the sample clean-up procedures are simplified, and the contamination of the capillary column and the detector caused by non-volatile matrices is effectively eliminated.

A main goal of the study is to apply the LINEX-DMI system into the determination of the pesticides that are sensitive to thermal decomposition and adsorption.

2.LINEX-DMI technique

The combination of the automatic liner-changing with the DMI is very effective for the determination of the pesticides in foods and the PCB in insulation oil. When applying the LINEX-DMI into a capillary GC/MS system, the contamination caused by the direct introduction of dirty samples can be prevented.

a) In the DMI system, a micro-vial is placed into the GC liner. (Figure 1).
b) A liquid or solid sample is added into the micro-vial. (size: 2mmI.D x 40mm length ; Figure 1).
c) The micro-vial is inserted and fixed on a narrow diameter of the liner. (Figure 1c).
d) The liner inserted with the micro-vial is automatically installed into the LINEX injection port by the FOCUS autosampler. (Figure 2, 3, 4)
e) A septum purge line can be performed using the LINEX head. (Figure 2) The PTV injection results in that the non-volatile matrices remain in the micro-vial, and prevents the contamination of the capillary column and MS. (Figure 1 e)

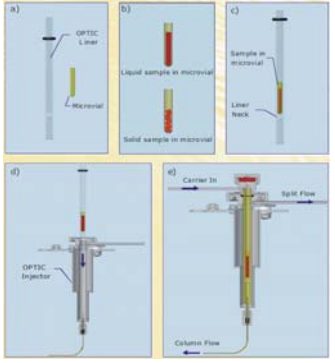

Figure 1. DMI technique

Figure 2. LINEX Head on OPTIC3 injector

Figure 3. LINEX Gripper on FOCUS robot

Figure 4. GC/MS system on LINEX system

3. Experimental

3.1. Chemicals and samples

- Internal standard component; Phenantrene - d10
- Standard sample 1; composite stock standard solution (1ng/μL) of 11 paraffinic hydrocarbons (n-C10, n-C12, n-C14, n-C16, n-C18, n-C20, n-C22, n-C24, n-C28, n-C30 and internal standard) was prepared in CHCl3 .
- Standard sample 2; composite stock standard solution (5ng/μL) of 15 pesticides (Fenobucarb, Bendaiocarb, Dimethoate, Lindane, Diazinon, Ethiofencarb, Carbaryl, Fenitrothion, Methiocarb, Aldrin, Fenthion, Dieldrin, Endrin, p,p'-DDT, Etofenprox and internal standard) was prepared in acetone.
- Standard sample 3; composite stock standard solution (1ng/μL) of 243 pesticides and internal standard was prepared in acetone.
- Extraction procedures are shown in Figure 10.

3.2. Instrumentation

3.2.1. LINEX-GC-MS analysis

3.2.1.1. LINEX system

- OPTIC3 injector (ATAS GL)
- FOCUS XYZ sample preparation robot (ATAS GL)

3.2.1.2. GC-MS system

- Model 6890 GC / 5973 MSD (Agilent Technologies)
- GC 353M / Varian 1200 (GL Sciences, Varian)
- Trace GC / Poraris Q (Thermo Electron)

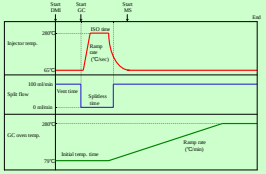

Figure 5. Optimized conditions in LINEX-DMI-GC/MS method

3.2.1.3. Column

- Inert Cap Pesticides, 0.25mmI.D. x 30M (GL Sciences)

3.3. Analytical conditions

3.3.1. Injection (DMI:Difficult Matrix Introduction)

Initial temp.: 65℃, ramp rate: 5℃/sec, final temp.: 280℃, iso time: 5min, cool down: 65℃

3.3.2. Oven program

- 79℃ (2min)-10℃/min-280℃ (5min)

4. Evaluation

An internal standard method and peak-area ratio of the pesticide sample to the internal sample was used in the evaluation of the LINEX-DMI system. The ratio is also utilized as a compensation factor to calibrate analytical reproducibility.

$$[\text{Peak area ratio}] = \frac{[\text{Pesticide peak area}]}{[\text{Internal standard peak area}]}$$

5. Results and Discussion

5.1 Reproducibility of analytical results for the normal hydrocarbons

The determination of the normal hydrocarbons C10 to C30 was repeatedly carried out using the internal standard method. The reproducibility (n=45) was calibrated with the compensation factor, and the results are shown in Figure 6.

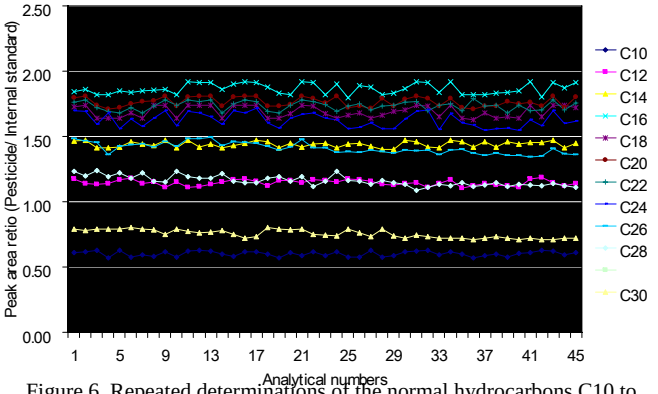

Figure 6. Repeated determinations of the normal hydrocarbons C10 to C30.

Table 1. Relative standard deviation of analytical results for the normal hydrocarbons

Hydrocarbon	C10	C12	C14	C16	C18	C20	C22	C24	C26	C28	C30
CV/%	3.31	1.92	1.59	2.14	2.62	2.06	2.00	3.33	2.97	3.33	4.14

As the above results, the performance of PTV injector used in the study is satisfactory.

5.2 Repeated determinations of the pesticide without the LINEX-DMI

The standard pesticide samples diluted with acetone were repeatedly introduced into the capillary GC/MS system without any exchanging of the liner. The data for each compound were compensated with internal standard method.

As shown in Figure 7, the analytical values decreased as the repeated times increased. The reasons for this are that thermal decomposition and adsorption occurred inside the liner due to the use of acetone which can lead to an increase in the activity of the liner and decrease in the recovery even if using a de-active liner.

During the analysis, the compounds 2, 6 and 7 shown in Table 2 were partly decompounded to the compounds a, b and c, respectively. Therefore, endrin ketone and endrin ketone increased as the repeated times increased due to thermal decomposition of the original compound (endrin).

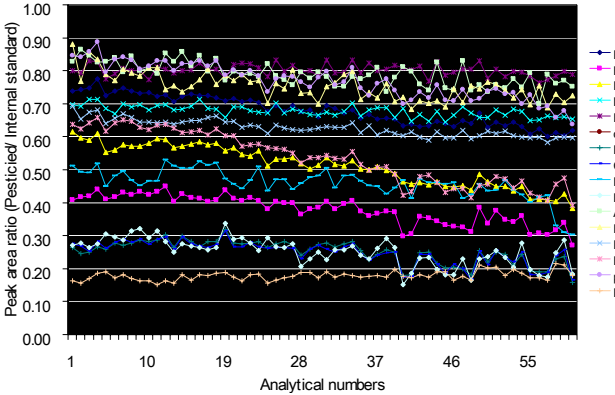

Figure 7. Repeated determinations of the pesticide

Table 2 Reproducibility (n=60) of the determination for standard sample 2

	Pesticide	CV(%)
1	Fenobucarb	6.57
2	Bendaiocarb	11.47
3	Dimethoate	11.96
4	Lindane	2.52
5	Diazinon	2.16
6	Ethiofencarb	14.08
7	Carbaryl	14.35
8	Fenitrothion	9.92
9	Methiocarb	16.73
10	Aldrin	4.56
11	Fenthion	5.58
12	Dieldrin	3.96
13	Endrin	14.81
14	p,p'- DDT	7.04
15	Etofenprox	7.77

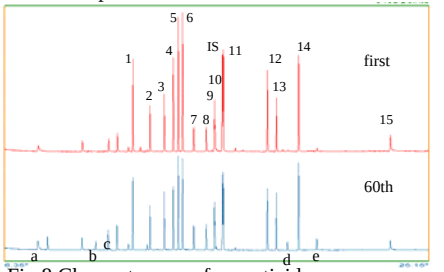

Fig.8 Chromatograms for pesticides

Table 3. Decompounded products

	Compounds
a	Bendaiocarb (decomposition)
b	Ethiofencarb (decomposition)
c	Carbaryl (decomposition)
d	Endrin ketone
e	Endrin aldehydo

5.3 Repeated determination of pesticides with the LINEX-DMI

When the repeated determinations (25 times) were carried out, one liner was used for 5 runs and automatically exchanged with a new one by the LINEX.

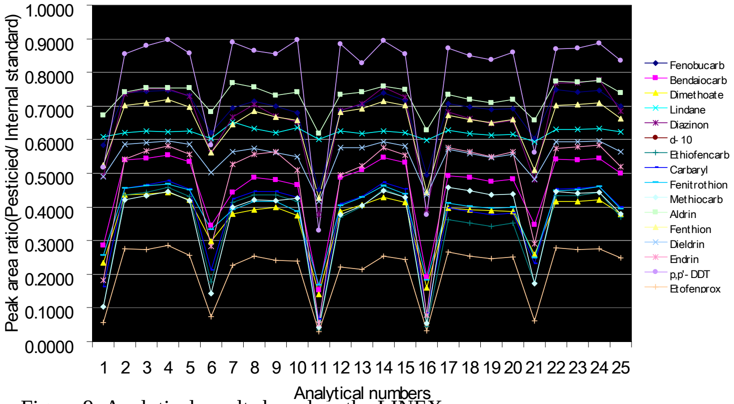

Figure 9. Analytical results based on the LINEX

Table 3. Average peak-area ratio (cycle using a same liner) and relative standard deviation (%)

	1 cycle		2 cycle		3 cycle		4 cycle		5 cycle		Cycle average CV(%)
	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	AVG	CV(%)	
Fenobucarb	0.74	0.84	0.70	2.07	0.71	2.97	0.70	1.13	0.73	3.07	2.84
Bendaiocarb	0.54	1.47	0.47	4.03	0.52	4.90	0.48	1.50	0.53	3.96	6.19
Dimethoate	0.43	2.15	0.39	2.97	0.41	4.29	0.39	1.06	0.41	4.83	4.73
Lindane	0.62	0.41	0.64	2.13	0.62	0.62	0.62	1.00	0.63	0.63	1.06
Diazinon	0.74	1.45	0.67	3.28	0.72	4.15	0.66	2.29	0.75	5.86	5.61
Ethiofencarb	0.44	2.77	0.43	3.09	0.41	8.52	0.35	2.31	0.42	8.01	8.35
Carbaryl	0.46	2.36	0.44	2.53	0.44	6.27	0.39	2.06	0.44	6.45	6.68
Fenitrothion	0.46	1.72	0.40	3.91	0.43	5.55	0.40	1.48	0.44	6.75	5.78
Methiocarb	0.43	2.76	0.42	2.72	0.41	7.48	0.45	2.36	0.43	7.53	2.99
Aldrin	0.75	0.84	0.75	2.26	0.75	1.42	0.72	1.35	0.77	2.29	2.17
Fenthion	0.71	1.28	0.66	2.48	0.70	2.09	0.66	1.32	0.69	3.05	3.06
Dieldrin	0.59	0.72	0.56	1.87	0.58	1.36	0.56	1.69	0.59	2.61	2.50
Endrin	0.56	2.83	0.54	4.58	0.54	6.64	0.56	1.93	0.56	5.34	2.48
p,p'- DDT	0.87	2.31	0.88	2.26	0.87	3.54	0.85	1.79	0.87	2.53	0.96
Etofenprox	0.27	4.58	0.24	4.54	0.23	7.95	0.25	3.38	0.27	5.18	6.82

As shown in Fig. 9, the recovery is poor for the first determination after changing the liner. This exhibits that it is necessary to condition a new liner. If removing the first data, the reproducibility was satisfactory for analytical results of each liner.

There are some considerable requirement and interest in throughput and matrix effect. The LINEX system is capable of making a variety of the liner-exchanging programs according to the requirements.

5.4 Preparation and determination based on the LINEX-DMI system

The preparation procedures of sesame are shown in Figure 10 (refer poster No. III-1-39C (SPW7-2) in detailed).

Sample 20 g
↓ (Sesame)
↓ add water (20 mL) swelling at 30 min
Extract with acetone/*n*-hexane (1:2) 100 mL
↓ mix 30 sec by homogeniser
↓ and mix 20 min by reciprocate shaker
Centrifuge 5 min. at 1200 x *g*
↓
Decant supernatant
↓ → → Residue was rextract with 50 mL
Evaporate
↓
Charge
↓ concentrated extract was
↓ diluted 20 mL with *Hex*
Elute
↓ eluted with 70 mL *ACN*
↓
Evaporate
↓ redissolve in 2 ml acetone
↓
GC/MS analysis

Figure 10. The preparation procedures of sesame

Exchanging of the liner was carried out for each run. 5μl extract obtained by the above preparation procedures and 0.3-ppm standard sample were introduced into the GC system by the LINEX-DMI, respectively. Figure 11 shows the chromatograms for the repeated determinations. Figure 12 shows a photograph of the liner in which sesame refuse remained after injection. The reproducibility of the determination of pesticides is reported in Table 5.

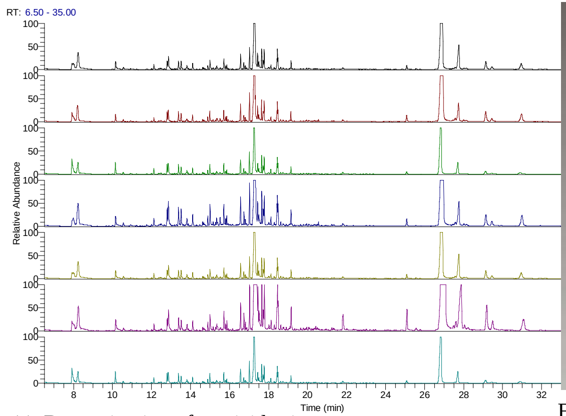

Figure 11. Determination of pesticides in sesame extract

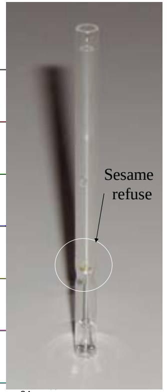

Figure 12. Sesame refuse remained in the liner

Table 5. Reproducibility of the determinations for the pesticides in sesame extract

Monocrotophos	8.73	12.55	Orbencarb	7.38	15.41	Butamifos	9.69	17.29	Pyridaphenthion	10.68	19.94
Pencycuron	13.75	12.68	Methiocarb	10.46	15.46	Napropamide	14.22	17.40	Bifenthrin	9.19	20.07
α-BHC	13.66	12.82	Dichlofluanid	33.13	15.54	Flutolanil	10.11	17.47	EPN	12.66	20.11
Hexachlorobenzen (HCB)	10.38	12.89	Esprocarb	8.60	15.56	Chlorfenson (CPCBS)	19.35	17.49	Bromopropylate	15.98	20.13
Dimethoate	7.49	13.15	Marathion	6.97	15.59	Profenofos	24.67	17.60	Bifenox	15.78	20.44
Ethoxyquin	13.16	13.21	Quinoclamin (ACN)	13.66	15.64	*p,p'*-DDE	9.43	17.67	Cyhalothrin	13.17	20.9
β-BHC	10.17	13.39	Chlorpyriphos	12.81	15.71	Dieldrin	9.30	17.74	Pyrazophos	10.89	21.26
Atrazine	11.16	13.41	Aldrin	11.11	15.73	*o,p'*-DDD	14.66	17.80	Fenarimol	9.68	21.27
γ-BHC	14.30	13.54	Thiobencarb (Benthiocarb)	5.80	15.74	Cyproconazole	12.48	18.05	Pyridaben	12.43	22.04
Cyromazine	14.23	13.55	Dimethylvinphos-*Z*	6.03	15.76	Isoxathion	36.73	18.06	Halfenprox	18.12	22.88
Propyzamide	5.38	13.75	Diethofencarb	5.21	15.79	Endrin	25.08	18.15	Fenvalerate	17.52	24.00
Chlorothalonil (TPN)	11.52	13.90	Parathion	15.21	15.88	Chlorobenzilate	15.24	18.31	Flvalinate	15.17	24.15
δ-BHC	7.15	14.12	Diphenamid	6.83	16.19	Nonachlor-*cis*	17.05	18.44			
Ethiofencarb	18.10	14.49	Pendimethalin	26.52	16.36	*p,p'*-DDD	17.60	18.48			
Benfuresate	3.34	14.63	Fipronil	11.16	16.47	*o,p'*-DDT	16.35	18.51			
Terbucarb (MBPMC)	5.80	14.69	Penconazole	10.83	16.48	Mepronil	19.81	18.76			
Propanil (DCPA)	6.15	14.71	Isofenphos	13.48	16.54	Chlornitrofen (CNP)	14.40	18.95			
Methylparathion	6.60	14.91	Heptachlorepoxide-*trans*	8.35	16.59	Propiconazole	21.82	19.04			
Alachlor	8.24	14.91	Procymidone	11.83	16.75	Pyriminobac-methyl-*E*	20.39	19.12			
Tolclofos-methyl	5.44	14.92	Methoprene	13.85	16.87	*p,p'*-DDT	17.45	19.17			
Heptachlor	8.45	15.02	Methidathion (DMTP)	10.63	16.98	Lenacil	11.48	19.19			

6. Conclusion

The Contamination of the capillary column and the MS detector can be prevented by the application of the LINEX-DMI system.

The Ministry of Health, Labour and Welfare has established general provisions concerning the introduction of the “positive list” system for residual agricultural chemicals in foods in the year. For this, we can provide a methodology that is capable of achieving more effective and simpler preparation, higher throughput and reliability.