Analysis of Organochlorine Pesticides (OCPs) present in *Ayurvedic Churnas* using Multi Dimensional Gas Chromatograph Mass Spectrometer (MDGC/GCMS)

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1: Introduction

Herbal medicine, also known as alternative medicine, is used to prevent and treat various ailments. Various herbs are known for having amazing medicinal properties. Use of herbs has been practiced for several centuries to cure a number of illness. Herbal medicine is one of the oldest forms of health care in India. Today, people around the globe are giving preference to alternative medicines such as *Ayurveda*, Naturopathy, Homeopathy and herbal medicine. Herbal medicines are safe, non-allergic and cost effective in comparison to Allopathic medicines.

The use of traditional Herbal medicines has started increasing from a past few decades and hence the safety has become a major concern for health authorities and for general public. The World Health Organization (WHO) has developed the strategies on traditional medicines. In India, The Govt. of India has formed a Department AYUSH (Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy) in Nov. 2003. AYUSH has come out with protocols[1] for pesticide residue analysis.

In this article, we have evaluated Herbal *Churnas* (powder) available in medical stores for analysis of Organochlorine Pesticides (OCPs) residue by developing a method on Multi Dimensional Gas Chromatography Mass Spectrometry (MDGC/GCMS) in Negative Chemical Ionization (NCI) mode. MDGC/GCMS helps in reducing matrix interference within one single run. This helps in high-throughput and multi component analysis. Using NCI in Single Ion Monitoring (SIM) mode further enhances specificity and sensitivity.

2: Method of Analysis

2-1: Extraction of OCPs from herbal *Churnas*

Herbal *churnas* namely Nityam *Churna*, Sitopaladi *Churna* and Hingashtaka *Churna* were procured from local medical stores and the pesticides were extracted from the *Churnas* by the Liquid Liquid Extraction method as per the following flow chart. The *Churnas* were spiked by known quantity of standard OCPs and then extracted.

Weigh 15g of *Churna* and add 15mL of 1% CH_3COOH in CH_3CN.
Add 75µL of OCP stock solution (each at 20ppm in Toluene)
Allow it to stand for at least 2 hours.

↓

Shake well, add 3g $MgSO_4$ + 1.5g NaOAc + 10g NaCl.
Shake well till the mixture mixed properly using Vortex mixture.

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Shake well and centrifuge at 1000rpm for 3mins.

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Add 200mg of Primary-Secondary Amine + 250mg of $MgSO_4$ + 50mg C18.
Shake well.

↓

Centrifuge at 1000rpm for 3mins.

↓

Take 2mL aliquot of extract and purge with slow stream of Nitrogen gas till 0.1mL of sample remains.

↓

Reconstitute to 1mL with Toluene. Used this extract as sample for analysis.

2-2: Analysis by MDGC/GCMS

The analysis of samples were carried out by MDGC/GCMS-QP2010 Ultra as per the conditions given in Table 1. MDGC is equipped with multi-Deans switching system[2], which allows the unresolved components of the mixture to introduce in the second GC column for further separation and then detection by MS in NCI mode. Figure 1 shows the working for multi-Deans switching system used for heart-cut. Figure 2 shows the photograph of Multi Dimensional GC/GCMS system.

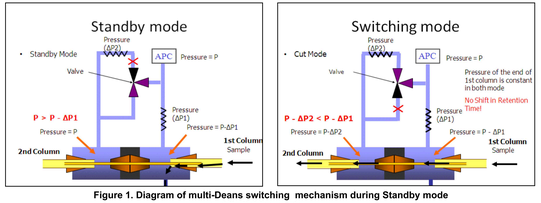

Figure 1. Diagram of multi-Deans switching mechanism during Standby mode and Switching mode

Figure 2. Multi-Dimensional GC/GCMS system

Key Features of MDGC/GCMS-QP2010Ultra

High-Speed Scanning Control (Advanced Scanning Speed Protocol, ASSP™)
High-Speed Scan Rate 20,000 u/sec
Overdrive Lens (Noise Elimination Technology)
Energy Saving- Ecology mode

Table 1. Analytical Condition for MDGC/GCMS

• 1st GC (GC-2010 Plus)			
Column	Rtx-5Sil MS (30m L x 0.25mm I.D. x 0.25µm)		
Column oven program	Ramp rate	Temperature	Hold time
		110°C	5 min
	15°C/min	200°C	0 min
	8°C/min	300°C	5 min
Injector temperature	280°C		
Detector temperature	300°C		
Detector Current	0.5nA		
APC1 Pressure	200 KPa		
Detector	ECD (Electron Capture Detector)		
• 2nd GCMS (GCMS-QP2010 Ultra)			
Column	Stx-CLPesticide (30m L x 0.32mm I.D. x 0.5µm)		
Column oven program	Ramp rate	Temperature	Hold time
		150°C	0 min
	5°C/min	250°C	5 min
	10°C/min	300°C	2 min
Ion source temperature	200°C		
Interface temperature	300°C		
Additional heater	250ºC		
Ionization mode	NCI (Negative Chemical Ionization)		
Acquisition mode	SIM (Selective Ion Monitoring)		
Carrier gas	Helium		
Reagent gas	Methane		

3: Results

First instrument was used as GC-ECD followed by GCMS in NCI mode with SIM. Figures 3 and 4 show the GC-ECD chromatogram and TIC of OCPs. Table 2 gives the results of GCMS analysis. Figure 5 gives the switching program for heart-cut of GC-ECD chromatogram where matrix interference were observed. These heart-cuts were switched to NCI with different switching timings for checking the presence of residual pesticide in matrix. Table 3 displays the quantitative results of pesticide residues present in *Churnas*.

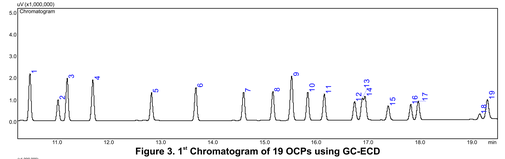

Figure 3. 1st Chromatogram of 19 OCPs using GC-ECD

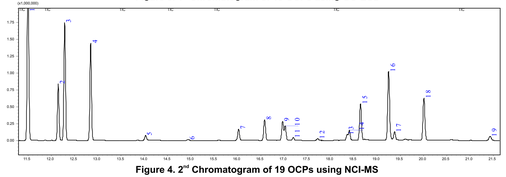

Figure 4. 2nd Chromatogram of 19 OCPs using NCI-MS

Table 2. Results of GCMS analysis for OCP standard

ID	Compound name	%RSD (n=6)		Linearity	LOD	LOQ
		RT(min)	Area	(10-250 ppb)	(ppb)	(ppb)
1	alpha.-BHC	0.013	0.48	0.998	0.34	1.13
2	gamma.-BHC	0.014	0.45	0.997	1.35	4.50
3	beta.-BHC	0.011	0.22	0.997	0.72	2.40
4	delta.-BHC	0.005	1.08	0.998	0.68	2.26
5	Heptachlor	0.013	0.27	0.998	16.28	54.27
6	Aldrin	0.011	0.11	0.998	10.63	35.44
7	Heptachlor-epoxide	0.002	0.43	0.998	14.78	49.26
8	trans-Chlordane	0.007	0.11	0.995	13.93	46.43
9	cis-Chlordane	0.003	0.14	0.996	19.59	65.30
10	p,p'-DDE	0.008	0.07	0.998	32.95	109.83
11	Endosulfan-I	0.006	0.12	0.998	32.03	106.78
12	Dieldrin	0.005	0.15	0.996	45.42	151.40
13	Endrin	0.011	0.17	0.996	7.65	25.49
14	p,p'-DDD	0.004	0.16	0.999	7.65	25.49
15	Endosulfan-II	0.013	0.11	0.997	7.45	24.84
16	p,p'-DDT	0.013	0.05	0.998	4.65	15.50
17	Endrin Aldehyde	0.005	0.11	0.998	26.40	87.99
18	Endosulfan sulfate	0.005	0.09	0.997	2.57	8.55
19	Endrin ketone	0.011	0.10	0.999	11.20	37.06

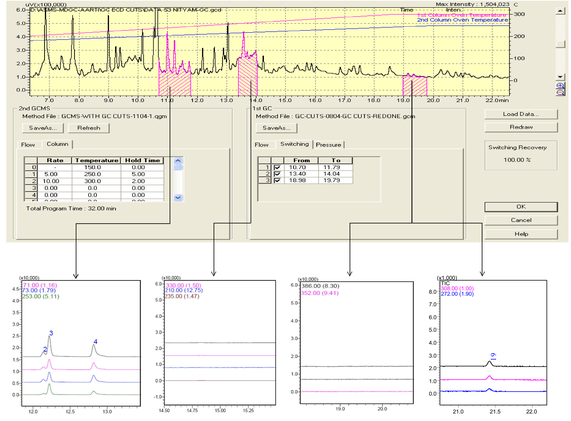

Figure 5. Switching program (Nityam *Churna* sample) for heart-cut to NCI-MS displaying mass chromatogram of gamma, beta, delta-BHC, Aldrin, Endosulfan sulfate and Endrin ketone respectively

Table 3. Quantitative results in mg/kg for different *Churnas* using 2nd GCMS

ID	Compound Name	RT (min)	AYUSH Limit (ppm)	Nityam *Churna*		Sitopaladi *Churna*		Hingashatak *Churna*	
				Conc. (ppm)	% Recovery	Conc. (ppm)	% Recovery	Conc. (ppm)	% Recovery
2	gamma.-BHC	12.08	0.60	0.03	109.75	0.02	109.75	0.05	113.64
3	beta.-BHC	12.32	0.30	0.03	107.07	ND	107.07	0.01	84.91
4	delta.-BHC	12.76		0.04	91.27	ND	91.27	0.04	95.64
6	Aldrin	14.80	0.05	ND	106.16	ND	106.16	ND	101.61
18	Endosulfan sulfate	19.95	3.00	ND	81.36	ND	81.36	ND	94.36
19	Endrin ketone	21.47	-	0.04	104.77	ND	104.77	0.03	110.08

4: Conclusion

- GCMS in NCI mode gave excellent %RSD for all 19 OCPs for retention time and area with good linearity.
- Multi Dimensional GC/GCMS is useful in reducing matrix effect by heart-cuts using multi-Deans switch and can be extended for testing herbal medicines.
- Analysis of residues in complex matrix can be carried out using MDGC/GCMS without any interference; so that false positive & false negative results are avoided.
- The *Churnas* tested were found to have pesticides within permissible limits as per AYUSH guideline.

5: References

[1] Protocol for testing guideline for Ayurvedic, Siddha and Unani medicines in Nov.2003, chapter 2.5.1., Dr. D.R. Lohar, Director, Department of AYUSH, Government of India.

[2] Application note SCA-180-017 Introduction of MDGC solution (Shimadzu Europa GmbH).