ANALYSIS OF PESTICIDE RESIDUES IN DRINKING WATER AS PER BUREAU OF INDIAN STANDARDS USING THE AGILENT 7000 GC/MS/MS WITH PESTICIDES ANALYZER

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ABSTRACT

An Agilent 7890B GC with 7000C MSD method for the analysis of residual pesticides in drinking water was developed, validated and successfully implemented for routine analysis as per the Bureau of Indian Standards (BIS). The method used the multi-mode inlet in PTV solvent vent mode, retention time locking and concurrent backflushing for the time effective elimination of less volatile matrix components from the GC column, which reduced cycle time and also prevented contamination of the MS source. This method used a simple sample preparation technique of liquid liquid extraction. Method detection limits of 5 ng/L were achieved for all pesticides, with linearity correlation coefficients > 0.995.

INTRODUCTION

The presence of organic contaminants in the environment are a result of pollution from various anthropogenic activities [1]. Pesticides, generated by the intensification of agriculture, are regarded as some of the most dangerous contaminants of the environment, despite their numerous merits.

Not only are they toxic, they are also mobile and capable of bioaccumulation. In addition, they can take part in various physical, chemical and biological processes.

Many of these pesticides are characterized by a strong persistence which explains their wide presence in the environment [2-4]. Due to their physicochemical characteristics and extensive use, many of these pesticides are common in surface water and a growing number of aquifers. Their presence in water is considered a potential risk not only for drinking water quality and human health, but also for ecosystems [3].

India is one of the largest pesticide consumers in the world. Due to the possible risks associated with pesticide use, water needs to be monitored. The Bureau of Indian Standards (BIS) sets regulatory limits for residual pesticides at trace levels (sub ppb) in drinking water (IS 10500) [5], packaged drinking water (IS 14543) [6] and natural mineral water (IS 13428) [7].

This study describes a method to determine pesticide residues at trace levels in water using liquid-liquid extraction and analysis using large volume injection (LVI) on the Agilent 7000C GC/MS/MS. Twenty eight pesticides were analyzed in water samples, including isomer and metabolite compounds. This technique is based on retention time locking and back flushing mode [8-9]. A method detection limit of 5 ng/L and quantitation limit of 10 ng/L were determined for each pesticide, with correlation coefficients > 0.999 (10 - 100 ng/mL). Parameters for the method validation were established by the European Commission (SANCO and SANTE) [10].

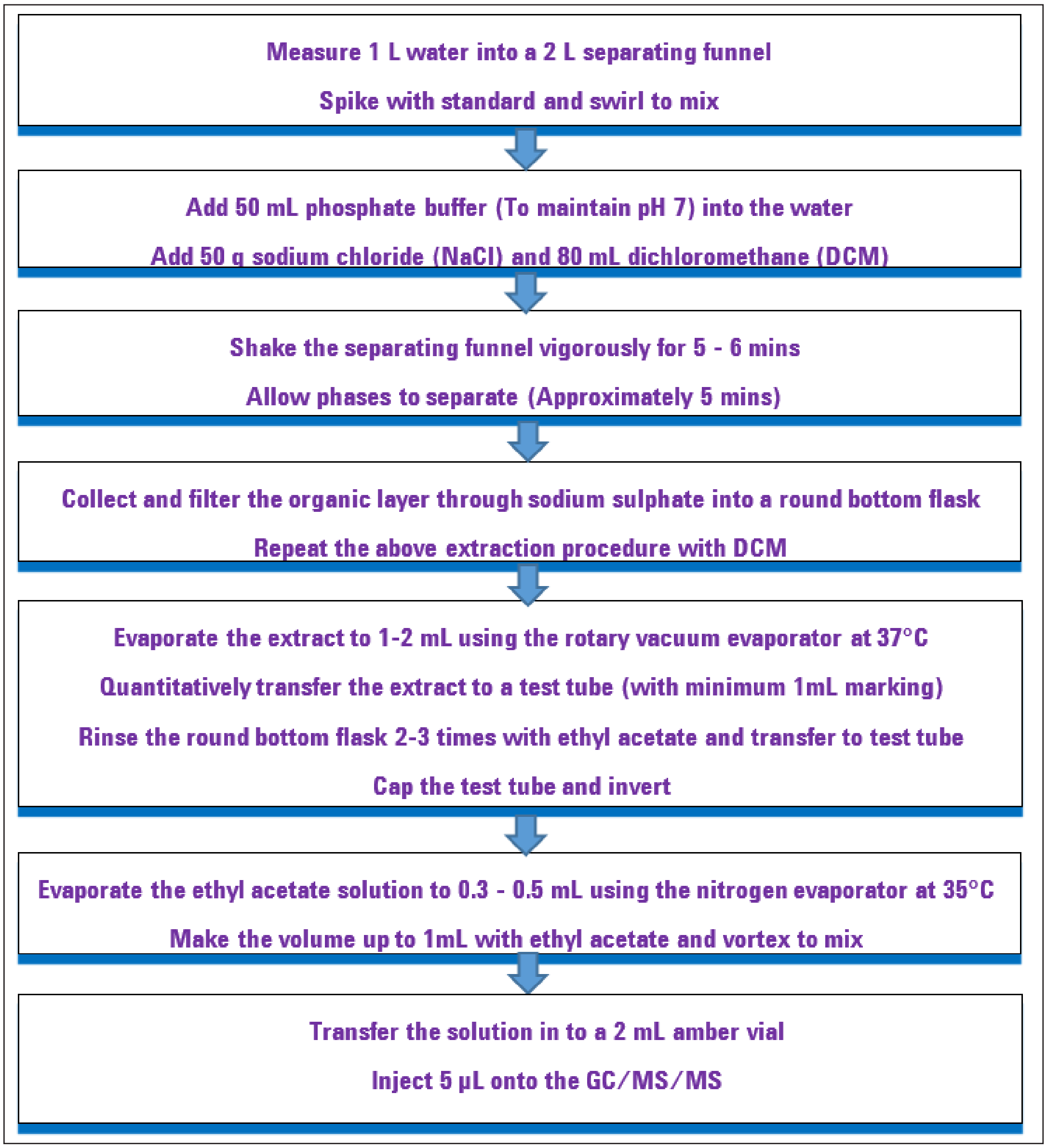
ANALYTICAL TECHNIQUE

Reagents and chemicals

Ethyl acetate and Dichloromethane (HPLC grade) were purchased from J.T. Baker. Water was purified in-house with a Milli-Q system (Millipore, Tokyo, Japan). Reagent grade anhydrous sodium sulfate, K2HPO4, sodium hydroxide and hydrochloric acid were obtained from Sd Fine Chemicals, Mumbai, India. All pesticides were of greater than 99% purity and were obtained from Sigma-Aldrich (USA). Stock solutions of individual pesticides at 100µg/mL were prepared by dissolving each pesticide standard in ethyl acetate and storing the solution at -20°C.

Preparation of Calibration Standards and Extracted Calibration Standards

Working standard solutions were prepared by diluting the stock solutions to 10µg/mL in ethyl acetate. Appropriate aliquots were taken and further diluted with ethyl acetate to give a series of calibration standard solutions with concentrations of 10 ng/mL, 20 ng/mL, 50 ng/mL, 75 ng/mL and 100 ng/mL. Extracted calibration standards were prepared by spiking blank water samples (1 L) with the working standard solution, prior to extraction, to give concentrations of 10 ng/L, 20 ng/L, 50 ng/L, 75 ng/L and 100 ng/L. After extraction and reconstitution (1 L to 1 mL) these equate to concentrations of 10 ng/mL, 20 ng/mL, 50 ng/mL, 75 ng/mL and 100 ng/mL.



Measure 1 L water into a 2 L separating funnel

Spike with standard and swirl to mix

Add 50 mL phosphate buffer (To maintain pH 7) into the water

Add 50 g sodium chloride (NaCl) and 80 mL dichloromethane (DCM)

Shake the separating funnel vigorously for 5 - 6 mins

Allow phases to separate (Approximately 5 mins)

Collect and filter the organic layer through sodium sulphate into a round bottom flask

Repeat the above extraction procedure with DCM

Evaporate the extract to 1-2 mL using the rotary vacuum evaporator at 37°C

Quantitatively transfer the extract to a test tube (with minimum 1mL marking)

Rinse the round bottom flask 2-3 times with ethyl acetate and transfer to test tube

Cap the test tube and invert

Evaporate the ethyl acetate solution to 0.3 - 0.5 mL using the nitrogen evaporator at 35°C

Make the volume up to 1mL with ethyl acetate and vortex to mix

Transfer the solution in to a 2 mL amber vial

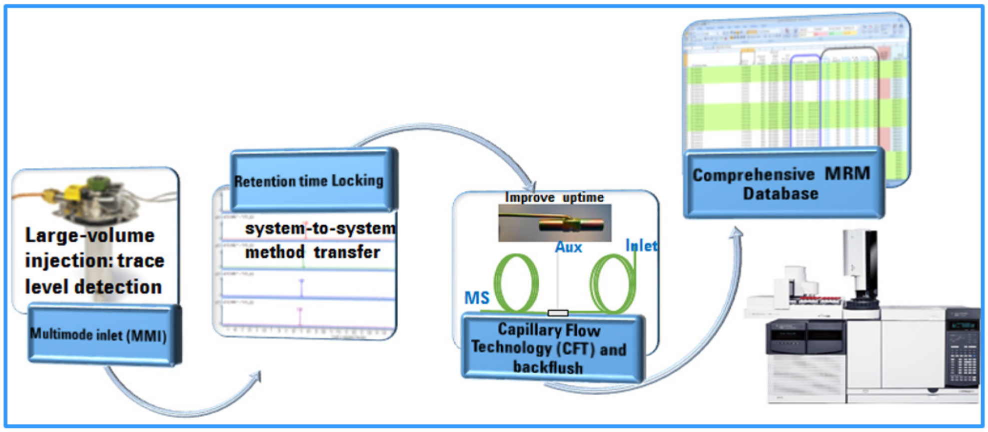
Inject 5 µL onto the GC/MS/MS

Instrumentation

Agilent 7890B Gas Chromatograph

Agilent 7000C Mass Selective Triple Quadrupole Detector

Pesticides Analyzer



GC/MS/MS Operating Conditions

GC Conditions	Agilent 7890B
Columns	1. HP-5ms: 15 m x 0.25 mm x 0.25 µm 2. HP-5ms: 15 m x 0.25 mm x 0.25 µm
Inlet	Agilent Multimode Inlet (MMI)
Split Mode and Ratio	Solvent vent mode (purge flow to split vent 60 mL/min at 2.62 psi & vent 25mL/min at 14.31 psi for 0.12 min)
Inlet Temperature	85°C (0.12 min), 600°C/min to 325°C
Oven	60°C (1 min), 40°C/min to 120°C, 5°C/min to 310°C
Carrier Gas	99.9995% Helium constant flow mode at 1.5 mL/min (2 psi during backflush) for column 1 (In front MMI, Out Aux EPC). Column 2 flow 1.7mL/min (In Aux EPC, Out MSD). Aux EPC pressure: 3.0 psi during run, 50 psi during backflush.
Run Time	40 minutes

MS Conditions	Agilent 7000C
Quadrupole Temperature	180°C
Ion Source Temperature	300°C
Transfer Line Temperature	280°C
Acquisition Mode	EI MRM

RESULTS AND DISCUSSIONS

The selectivity or specificity of the analytical method for the determination of 28 pesticides in water is shown in Figure 1 and Table 1. The calibration curve for each pesticide was plotted using peak area against concentration of pesticide in both the water matrix and aqueous solvent. They were linear with correlation coefficients (r2) > 0.995, as shown in Table 1. Method detection limits of 5 ng/L were determined for all pesticides, by using the signal to noise (S/N) ratio of 3:1 for the strongest mass transition (MRM) with respect to the background noise obtained from the blank sample and method quantitation limits (MQL) of 10 ng/L were determined similarly by considering the signal to noise ratio (S/N) ratio of 10:1. Recovery and precision studies were carried out both inter-day and intra-day at three different spiked concentration levels (10 ng/L, 20 ng/L and 50 ng/L). Recoveries of the 28 pesticide residues were in the range of 88 - 99%, with RSDs of less than 8%. Results are shown in Table 1.

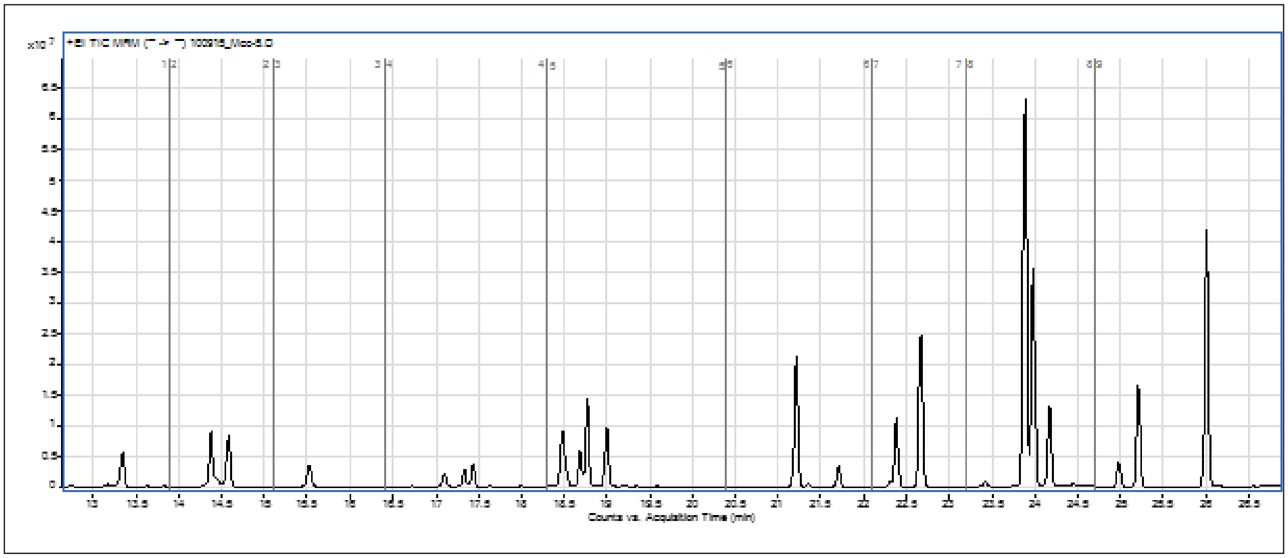


Figure 1. Extracted pesticides standard TIC.

Pesticide	R.T Min	Spiking Level ng/L	% Recovery (n=7)	% RSD (n=7)	MRM	Linearity (Aqueous)	Linearity (Matrix)
Monocrotophos	13.162	10.0	92.18	5.78	127.1>109.0 (10) 127.0>95.0 (15)	0.9994	0.9992
		20.0	94.24	4.82			
		50.0	94.71	4.39			
Phorate	13.278	10.0	88.29	6.89	121.0>47.0 (30) 121.0>65.0 (10)	0.9985	0.9956
		20.0	89.64	7.45			
		50.0	92.57	5.81			
Alpha-BHC	13.343	10.0	96.84	3.89	216.9>181.0 (5) 218.9>183.0 (5)	0.9994	0.9994
		20.0	97.69	5.82			
		50.0	98.58	4.60			
Atrazine	14.433	10.0	95.71	4.91	214.9>58.1 (10) 214.9>200.2 (10)	0.9995	0.9991
		20.0	96.64	3.86			
		50.0	96.79	3.73			
Beta-BHC	14.579	10.0	96.60	3.81	216.9>181.1 (5) 181.0 >145.0 (15)	0.9993	0.9991
		20.0	96.91	4.01			
		50.0	98.12	3.66			
Gamma-BHC	14.581	10.0	97.42	3.66	216.9 >181.0 (5) 181.0>145.0 (15)	0.9989	0.9990
		20.0	97.29	3.86			
		50.0	98.94	2.57			
Delta-BHC	15.517	10.0	95.35	3.53	217.0>181.1 (5) 181.1>145.1(15)	0.9997	0.9994
		20.0	97.69	3.39			
		50.0	97.77	3.11			
Paraoxon-methyl	15.548	10.0	90.11	5.82	229.9>106.0 (15) 229.9>200.1 (5)	0.9986	0.9974
		20.0	90.52	4.60			
		50.0	93.60	4.91			
Parathion-methyl	17.103	10.0	91.10	4.82	262.9>109.0 (10) 125.0>47.0 (10)	0.9988	0.9987
		20.0	91.92	4.29			
		50.0	92.67	4.22			
Malaoxon	17.333	10.0	89.55	5.96	127.0>99.0 (5) 127.0>55.0 (5)	0.9991	0.9992
		20.0	93.51	5.88			
		50.0	94.39	5.13			
Alachlor	17.434	10.0	94.76	3.39	181.1>160.2 (10) 160.0>132.1 (10)	0.9996	0.9986
		20.0	95.01	3.11			
		50.0	96.53	2.50			
Phorate sulfoxide	18.480	10.0	90.22	4.98	153.0>96.9 (10) 96.9>64.9 (20)	0.9997	0.9994
		20.0	89.10	5.19			
		50.0	92.54	4.23			
Aldrin	18.525	10.0	96.28	3.57	262.9>192.9 (35) 254.9>220.0 (20)	0.9992	0.9996
		20.0	98.12	2.94			
		50.0	98.58	2.22			
Malathion	18.689	10.0	93.35	3.66	126.9>99.0 (5) 172.9>99.0 (15)	0.9984	0.9971
		20.0	94.20	3.58			
		50.0	94.85	2.88			

Pesticide	R.T Min	Spiking Level ng/L	% Recovery (n=7)	% RSD (n=7)	MRM	Linearity (Aqueous)	Linearity (Matrix)
Phorate sulfone	18.770	10.0	88.49	6.43	153.0>97.0 (10) 124.9>96.9 (5)	0.9965	0.9969
		20.0	91.26	6.19			
		50.0	91.71	5.80			
Chlorpyrifos	18.998	10.0	96.83	3.73	196.9>169.0 (15) 198.9>171.0 (15)	0.9992	0.9989
		20.0	97.85	2.50			
		50.0	98.56	2.44			
o,p-DDE	21.207	10.0	97.08	3.58	246.0>176.2 (30) 248.0>176.2 (30)	0.9992	0.9999
		20.0	97.87	2.99			
		50.0	98.90	2.52			
α-Endosulfan	21.351	10.0	93.48	4.75	194.9>159.0 (5) 194.9>160.0 (5)	0.9997	0.9997
		20.0	95.92	4.39			
		50.0	96.21	3.99			
Butachlor	21.709	10.0	94.80	3.86	176.1>147.1 (10) 188.1>160.2 (10)	0.9990	0.9991
		20.0	96.55	3.22			
		50.0	97.69	3.09			
Dieldrin	22.307	10.0	95.86	4.33	262.9>191.0 (35) 277.0>241.0 (5)	0.9997	0.9998
		20.0	97.01	2.89			
		50.0	97.52	3.24			
p,p-DDE	22.381	10.0	96.86	3.79	246.1>176.2 (30) 315.8>246.0 (15)	0.9999	0.9995
		20.0	97.54	3.11			
		50.0	98.01	2.80			
o,p-DDD	22.669	10.0	97.32	2.91	235.0>165.2 (20) 237.0>165.2 (20)	0.9999	0.9999
		20.0	97.66	3.07			
		50.0	98.07	2.16			
β-Endosulfan	23.420	10.0	95.53	4.91	206.9>172.0 (15) 194.9>158.9 (10)	0.9989	0.9992
		20.0	96.27	4.66			
		50.0	96.92	2.49			
p,p-DDD	23.883	10.0	96.77	3.27	234.9>165.1 (20) 236.9>165.2 (20)	0.9999	0.9997
		20.0	97.51	2.33			
		50.0	98.39	2.19			
o,p-DDT	23.980	10.0	97.44	2.98	234.9>165.1 (20) 236.9>165.2 (20)	0.9998	0.9999
		20.0	98.76	2.55			
		50.0	98.99	2.42			
Ethion	24.167	10.0	96.85	3.14	230.9>129.0 (20) 230.9>175.0 (10)	0.9996	0.9994
		20.0	96.79	2.86			
		50.0	97.93	2.45			
Endosulfan sulfate	24.981	10.0	93.08	5.43	271.9>237.0 (15) 273.8 >238.9 (15)	0.9984	0.9983
		20.0	95.21	5.11			
		50.0	95.34	3.37			
p,p-DDT	25.212	10.0	96.88	3.81	235.0>165.2 (20) 237.0>165.2 (20)	0.9998	0.9999
		20.0	97.83	2.93			
		50.0	98.71	2.42			

CONCLUSIONS

The design of the Agilent 7000C Triple Quadrupole GC/MS enables pesticides to be determined at lower detection limits when combined with an inert sample path and GC column backflushing. The high sensitivity EI Extractor Ion Source with improved thermal characteristics delivers confident trace analysis even in complex matrices, and the Triple-Axis HED-EM Detector reduces neutral noise by the doubly off-axis position of the HED-EM. The simple, easy, economical and efficient method was developed for the determination of 28 pesticides in water. The method demonstrates good sensitivity, precision, accuracy and allows for rapid analysis. The results demonstrate that pesticide residues can be detected below the current maximum residue levels (MRL) required by the BIS specifications. Method detection limits of 5 ng/L and quantitation limits of 10 ng/L were achieved for all pesticides. The method is ideally suited for use in a regulatory laboratory for the determination of pesticides in surface, drinking and packaged drinking water.

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