

ENVIRONMENTAL ANALYSIS

HPLC ANALYSIS OF ISOPROTURON IN WATER USING ONLINE SAMPLE ENRICHMENT



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ABSTRACT

This application note demonstrates a selective and sensitive online enrichment method for the determination of Isoproturon in environmental and drinking water samples. Automated sample enrichment and cleanup were achieved using a 2 position/6 port valve with an Agilent Poroshell 120 SB-Aq column.



The Isoproturon was separated on an Agilent ZORBAX RRHD SB-C18 column and detected at 240nm with the 1260 Infinity DAD using the 60 mm high Sensitivity Max-Light cell. A high recovery of 99.2%, good reproducibility with relative standard deviations lower than 0.5% and a linear dynamic range up to 50 µg/L are observed, with a detection limit of 0.5 µg/L.

INTRODUCTION

The pollution of soil, ground, and surface water involves a serious risk to the environment and also human health by way of direct exposure or through residues in food and drinking water. Herbicide contamination in water bodies is monitored to mitigate adverse health effects.

A sensitive, rugged and economic method to determine Isoproturon at trace levels was developed using online sample enrichment. The sample is pre-concentrated on the trapping column in position A of the 2 position /6 port valve. The enriched sample is then eluted from the trapping column onto the analytical column for separation in position B of the valve. A sensitivity equivalent to that of LC/MS was achieved by using this Agilent valve solution.



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ANALYTICAL TECHNIQUE

Instrumentation:

The Agilent LC system consisted of the following modules:-

- Agilent 1260 Quaternary Pump (G1311B) as Pump A
- Agilent 1260 Infinity Micro Degasser (G1379B)
- Agilent 1260 Infinity Autosampler (G1329B) modified with a Multi draw kit (G1313-68711)
- Agilent 1260 Infinity Thermostat (G1330B)
- Agilent 1290 Binary Pump (G4220A) as Pump B
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Two-Position/ Six-Port column switching valve (5067-4646)
- Agilent 1260 Infinity Diode Array Detector (G4212B), with 60 mm Max-Light high sensitivity flow cell
- Software: OpenLAB ChemStation C.01.07

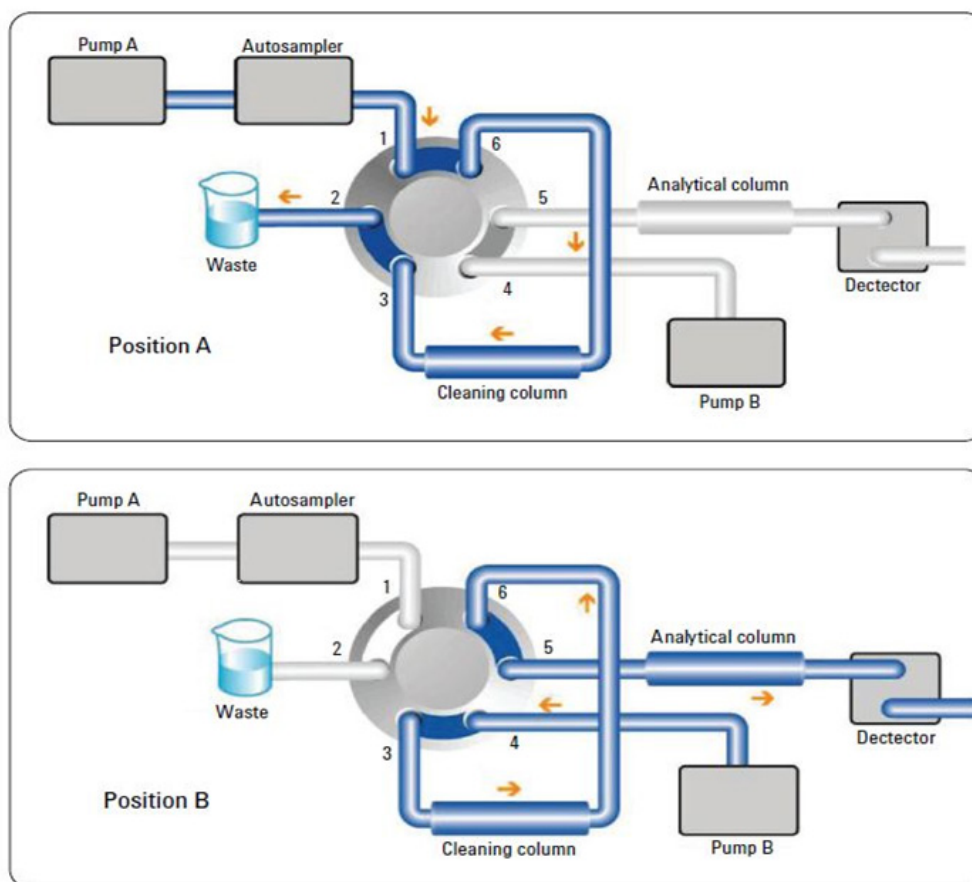


Figure 1. Schematic diagram of valve positions during online sample enrichment.

Chromatographic Conditions		
Enrichment/Cleaning Column:	Poroshell 120 SB-Aq 3.0 X 50 mm, 2.7 µm (p/n 689975-314)	
Analytical Column	Agilent ZORBAX RRHD SB-C18 3.0 x 100 mm, 1.8 µm (p/n 858700-302)	
Mobile Phase A	Milli-Q water	
Mobile Phase B	Acetonitrile	
Injection volume	1000 µL	
Enrichment/Loading conditions		
Flow rate	0.5 mL/min	
Gradient	Time (min)	% Acetonitrile
	0	0
	5.1	10
	6.0	10
	6.1	0
Stop time	no limit	
Elution and Analysis Conditions		
Flow rate	0.6 mL/min	
Gradient	Time (min)	% Acetonitrile
	0	20
	6.0	20
	9.5	90
	10.5	90
	11.0	20
Stop time	12 min	
Column temperature	40 °C on both sides	
Column switching		
0 min:	Load/Equilibrate (valve position 1)	
6 min:	Elute/Equilibrate (valve position 2)	
11 min:	Load/Equilibrate (valve position 1)	
Detection:	240 nm, 8 nm BW; Ref: No; PW > 0.062 s (20 Hz)	

Standards and Samples

Stock solutions were prepared in methanol and diluted in Milli- Q water to obtain calibration standards at 1.0, 2.5, 5.0, 10.0 and 50.0 µg/L. Tap water was used as a sample and spiked at 1, 10 and 50 µg/L to check the recoveries at low, mid and high points in the calibration ranges. A 50 µg/L standard was injected six times to check the RT and area repeatability.

RESULTS AND DISCUSSIONS

A limit of quantitation (LOQ) of 1.0 µg/L (S/N =10) and an LOD of 0.5 µg/L (S/N >3) were achieved for Isoproturon after online sample enrichment and UV detection at 240nm, as shown in Figure 2. An overlay of the calibration standards (1.0 – 50.0 µg/L) and a typical calibration are shown in Figure 3.

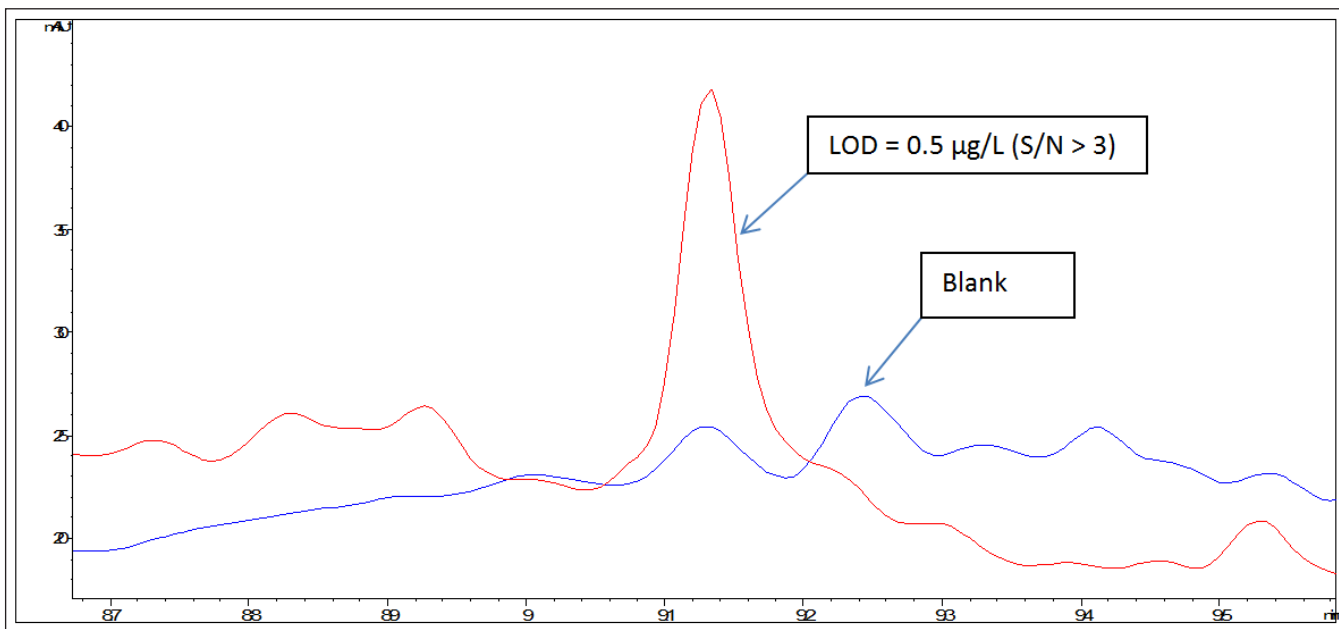


Figure 2. Chromatograms showing a Blank and Isoproturon at the LOD (0.5 µg/L).

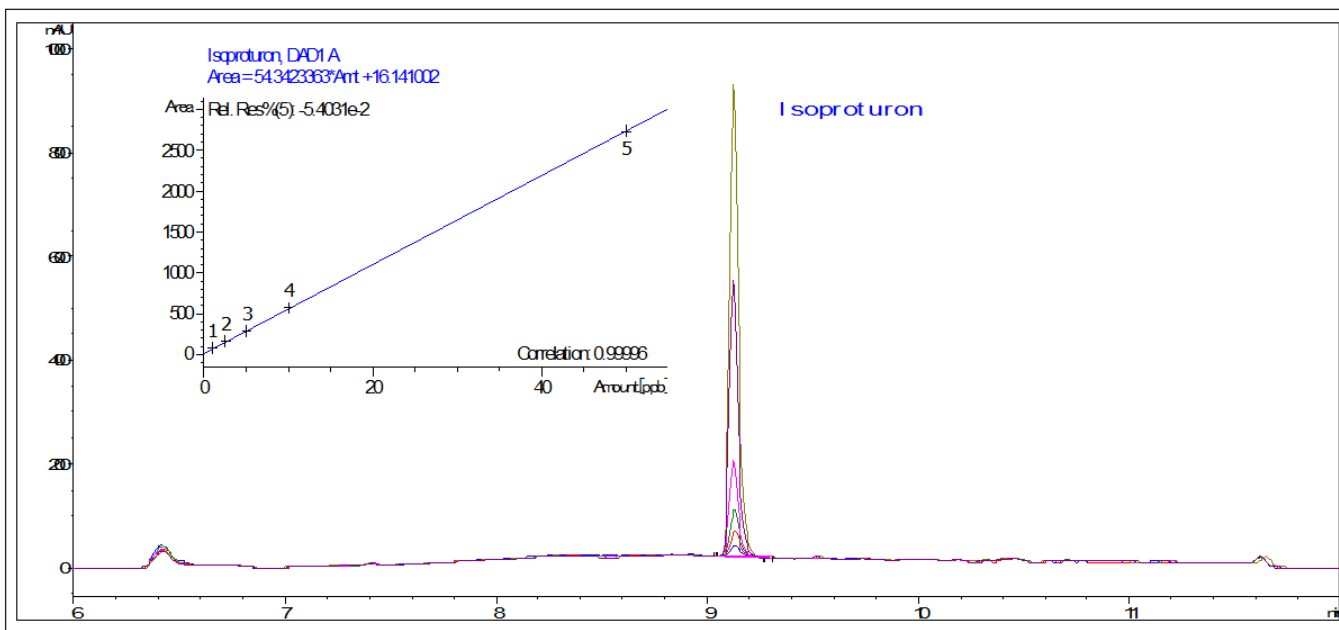


Figure 3. Overlay of chromatograms in the linearity range of 1.0 - 50.0 µg/L and the calibration curve showing R² = 0.999.

The precision of the method was tested using six replicate injections of the 50.0 µg/L standard. Figure 4 shows the overlay of the replicate injections. The retention time (RT) of Isoproturon was observed at 9.129 minutes with a RT RSD of 0.10% and an area RSD of 0.16%.

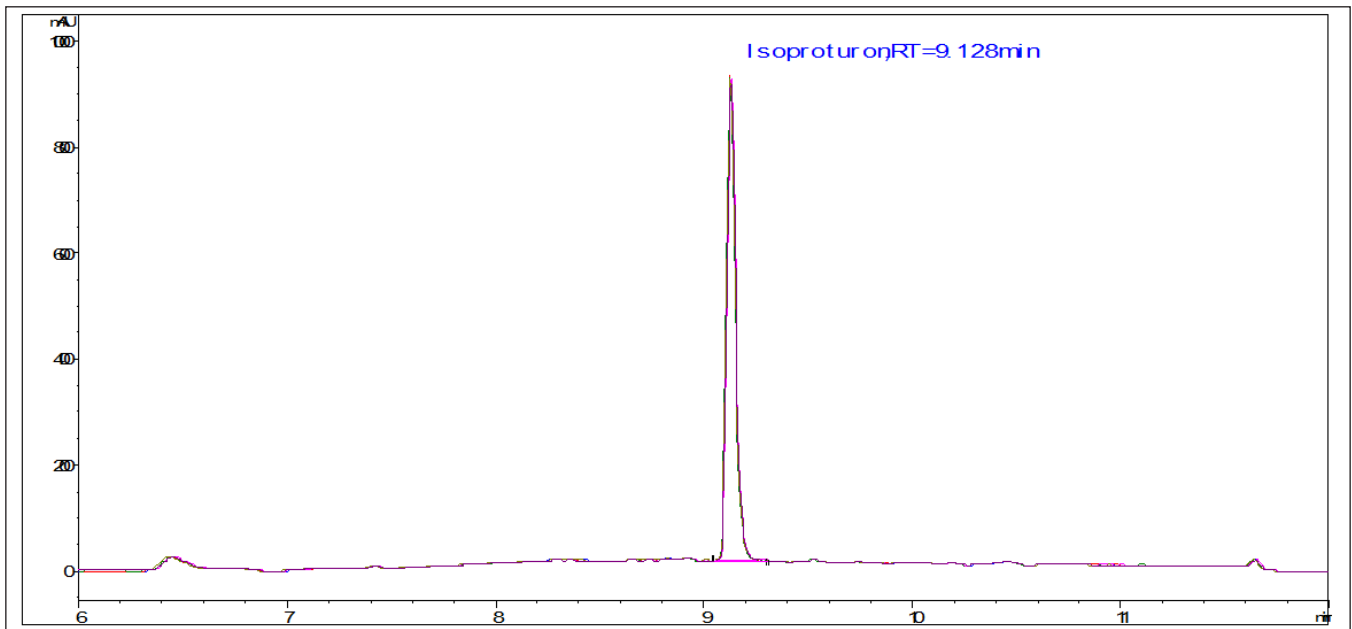


Figure 4. Overlay of chromatograms showing six replicate injections at 50.0 µg/L.

The precision of the method was tested using six replicate injections of the 50.0 µg/L standard. Figure 4 shows the overlay of the replicate injections. The retention time (RT) of Isoproturon was observed at 9.129 minutes with a RT RSD of 0.10% and an area RSD of 0.16%.

Spike Level (µg/L)	% Recovery	% Mean recovery
1.0 (Low)	97.48	99.24
10.0 (Mid)	100.26	
50.0 (High)	99.98	

Table 1. Percentage Recoveries across various levels of the linearity range.

CONCLUSIONS

According to the regulatory authority, the Bureau of Indian Standards (BIS), the maximum permissible concentration of Isoproturon in water is 9 µg/L. This is easily achieved using HPLC online sample enrichment and detection with the Agilent 1260 Infinity DAD. The automated sample preparation technique using a valve solution avoids laborious offline SPE and ensures unattended operation, whilst achieving excellent sensitivity equivalent to that of LC/MS.

REFERENCES

1. S. Kailasam, Trace Analysis of Chlorinated Herbicides in Water with Online Enrichment. A simple and rapid method with automated sample cleanup. Agilent Technologies publication 5990-6922EN, Nov 2012.



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