Application Note

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Improved Thermal Stability Leads to Retention Time Stability and Data Accuracy for the Analysis of Pesticides

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Abstract

Analyzing trace level pesticides can prove difficult due to heavy matrix and thermal stress potentially causing damage to gas chromatography (GC) column phases. The Agilent J&W 5Q series GC columns with increased thermal stability offer a robust solution to mitigate phase degradation and maintain retention time stability, even when stressed with heavy matrices such as soil. Additionally, the increased thermal stability of the Agilent J&W DB-5Q GC column greatly reduces the interfering column bleed ions that allow for cleaner analyte mass spectra and lead to improved library matching. This study illustrates the achievable reproducibility, data accuracy, and sensitivity of the analysis of pesticides when the Agilent 5977B gas chromatography/mass selective detector (MSD) and the Agilent 7010D triple quadrupole gas chromatography/mass spectrometry system (GC/TQ) systems are used.

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Introduction

The analysis of trace-level pesticides is required by many governmental regulatory authorities at varying concentrations and can include hundreds of target analytes. As many of the matrices tested are complex and the analytes active, a robust and inert gas chromatography method is required.[1] Heavy matrices can be difficult for analytical gas chromatography columns, and higher temperatures may be required to elute the matrix off of the column. To avoid damage from high temperatures and heavy matrix, a thermally stable and ultra inert analytical column phase is needed to successfully perform this analysis. The Agilent J&W 5Q series of gas chromatography columns provide the most thermally stable column available and offer inertness for difficult analytes.[2] The increased thermal stability not only dramatically reduces column bleed but also allows for less spectral interference at higher temperatures, leading to less need for background subtraction when searching analyte spectra against commercially available libraries.

While column bleed is a normal phenomenon, the increase of the baseline due to column bleed is an indication of the column phase breaking down and leading to retention time shifts. Retention time shifting can be especially difficult with the analysis of pesticides, since sensitive detectors such as mass spectrometers and triple quadrupole systems collected in selected ion mode (SIM) or multiple reaction monitoring (MRM) mode are required to achieve lower limits of detection. In both SIM and MRM collection methods, time segment windows are stated for analytes of interest, and if a retention time shifts earlier, it can move out of the collection window and require time to update the analytical collection method. By using a GC column with increased thermal stability, such as the Agilent J&W DB-5Q or the Agilent J&W HP-5Q, the column will be more robust to thermal and matrix damage. These columns will also maintain consistent retention times over long periods of analytical stress.

Retention time locking is a useful tool in the analysis of pesticides, as it can adjust column flow or pressure to normalize retention times, so that the retention times of the analytes are the same from system to system or column to column.[3,4] When columns with identical selectivity are interchanged, after a single injection of a test mix, the analysis can be relocked and the column flow adjusted, based on the initial calibration, to be identical as in the original method. This eliminates the need to constantly update retention times in analytical methods for SIM or MRM windows or data processing methods when replacing analytical columns.

When analyzing pesticides in heavy matrix, a guard column can serve two additional benefits. The guard column can help protect the analytical column from heavy matrix damage and can improve solvent and sample focusing. It is recommended to use nonpolar solvents when working with nonpolar column phases, such as the DB-5Q and HP-5Q columns. Recently it has become more common to prepare heavy matrices for pesticide analysis using QuEChERs-type preparations, with a final solvent of acetonitrile. Solvent-column phase polarity mismatch can lead to improper solvent focusing and wettability leading to improper peak shape, especially when working with columns that have low free silanol content on the deactivated surface of the fused silica. Use of a guard column can help to refocus the solvent and analytes to maintain proper peak shape.[5]

The Agilent 7010D GC/TQ uses the new Agilent High Efficiency Source (HES) 2.0 equipped with a novel dipolar RF lens that redirects carrier gas and low mass ions by > 95%, helping to reduce noise and extend instrument robustness while maintaining sensitivity.[6] When using DB-5Q and HP-5Q columns in conjunction with the HES 2.0, it is possible to increase the overall robustness of the workflow for the analysis of pesticides over a wide dynamic range of analyte concentrations.

Experimental

An Agilent 8890 GC coupled with an Agilent 5977B GC/MSD with an Inert Extractor Source was used for acquisition of data and MassHunter 10.0 Qualitative Analysis software was used for data analysis. NIST20 library was used to confirm peak identification. A retention gap of one meter was used as a guard column to help protect the analytical column from heavy matrix and to aid in solvent focusing.

A representative pesticide mixture, the Agilent pesticide checkout (part number 5190-0468) was used to monitor analytical performance. Calibration standards were prepared in dichloromethane (DCM) at concentrations ranging from 10 to 500 ppb, from the pesticide checkout standard.

The acquisition method was locked, using retention time locking, for the analyte chlorpyrifos-methyl at a retention time of 18.0 minutes, using ions 125, 286, and 288 for peak confirmation.

A composite mixture of soils extracted with DCM to monitor performance when stressed with a heavy matrix, which is a representative matrix residue that is typically encountered in the lab, was procured from Pace Analytical (Mt. Juliet, TN).

Table 1. GC parameters for the Agilent 8890 GC.

8890	
Inlet	300 °C, Pulsed splitless, 50 psi until 0.75 min
Purge Flow to Split Vent	50 mL/min at 0.7 min
Injection Volume	1.0 mL
Inlet Liner	Ultra Inert, split, low pressure drop (part number 5190-2295)
Gas Saver	On, 20 mL/min after 3 min
Septum Purge Flow	3 mL/min
Oven	80 °C (1.5 min), Ramp 40 °C/min to 120 °C, Ramp 5 °C/min to 310 °C (10 min)
Column	
Carrier Gas	Helium, 1.37 mL/min, Constant Flow
Column	– DB-5Q, 30 m × 0.25 mm, 0.25 µm (part number 122-5532Q) – Deactivated fused silica, 1 m, 0.25 mm id (part number 160-2255-1)
Inlet Connection	Split/splitless inlet (S/SL)
Outlet Connection	MSD

Table 2. MS parameters for the Agilent 5977 GC/MSD.

Parameter	Value
Model	5977B
Source	XTR
Mode	Scan (40 to 500 amu)
Solvent Delay	4.0 min
Source Temperature	300 °C
Quad Temperature	175 °C
Gain	1.0

Table 3. MS parameters for the Agilent 7010D GC/TQ.

Parameter	Value
Model	7010D
Source	HES
Mode	DMRM/Scan
Solvent Delay	4.0 min
Source Temperature	300 °C
Quad Temperature	175 °C
Gain	1.0

Table 4. Quantitative/qualitative transitions (dMRM based) for Agilent 7010D GC/TQ acquisition parameters.

			Quant			Qual		
Peak	Compound Name	RT (min)	Precursor Ion	Product Ion	CE	Precursor Ion	Product Ion	CE
1	Dichlorvos	5.917	109	79	5	109	47	15
2	Mevinphos	8.902	127	109	10	127	95	15
3	Ethalfluralin	13.368	276.1	105.1	35	276.1	202	20
4	Trifluralin	13.717	306.1	264	5	306.1	160	30
5	Atrazine	15.412	200.1	122.1	10	200.1	104	20
6	Lindane (γ-BHC)	15.604	181	145	15	181	109	30
7	Chlorpyrifos-methyl	17.928	286	93	25	286	270.9	20
8	Heptachlor	18.413	271.9	236.8	25	271.9	116.9	40
9	Malathion	19.614	173.1	99	15	173.1	117	10
10	Chlorpyrifos	19.797	196.9	168.9	15	196.9	107	40
11	Dieldrin	23.570	262.9	192.9	40	262.9	190.9	35
12	DDE, p,p'-	23.648	246	176.1	40	246	175.1	40
13	Hexazinone	26.687	171.1	71.1	15	171.1	85.1	15
14	Propargite	27.241	135.1	107.1	15	135.1	77.1	30
15	Leptophos	29.547	171	77.1	25	171	124.1	10
16	Mirex	30.198	271.9	236.9	15	271.9	116.9	40
17	Fenarimol	30.546	139	111	15	139	75	35
18	Coumaphos	32.053	362	109	15	362	81	40
19	Etofenprox	34.271	163.8	107.2	18	163.8	135.3	5
20	Deltamethrin	36.819	181.1	152.1	25	181.1	127.1	25

Results and discussion

The analysis of pesticides requires retention time stability as well as consistency from analytical column to analytical column. To compare the analytical performance of an Agilent J&W DB-5Q to an Agilent J&W DB-5ms UI GC column for the analysis of pesticides, a standard was first analyzed on a DB-5ms UI and the retention time locked for chlorpyrifos-methyl. The DB-5ms UI column was then replaced with a DB-5Q, and once the column was conditioned, the representative pesticide mix was again analyzed and relocked to the appropriate retention time for chlorpyrifos-methyl and analyzed again. Figure 1 demonstrates the identical selectivity of the DB-5ms UI to the DB-5Q phases and the ability to relock an existing analytical method when upgrading columns and compatibility with existing retention time locking libraries.

Additionally, when comparing the baseline of the DB-5Q to the DB-5ms UI column at the upper temperature limit of the chromatographic method, the increased thermal stability of the DB-5Q allows for a significant decrease in column bleed at upper temperature limits (Figure 1).

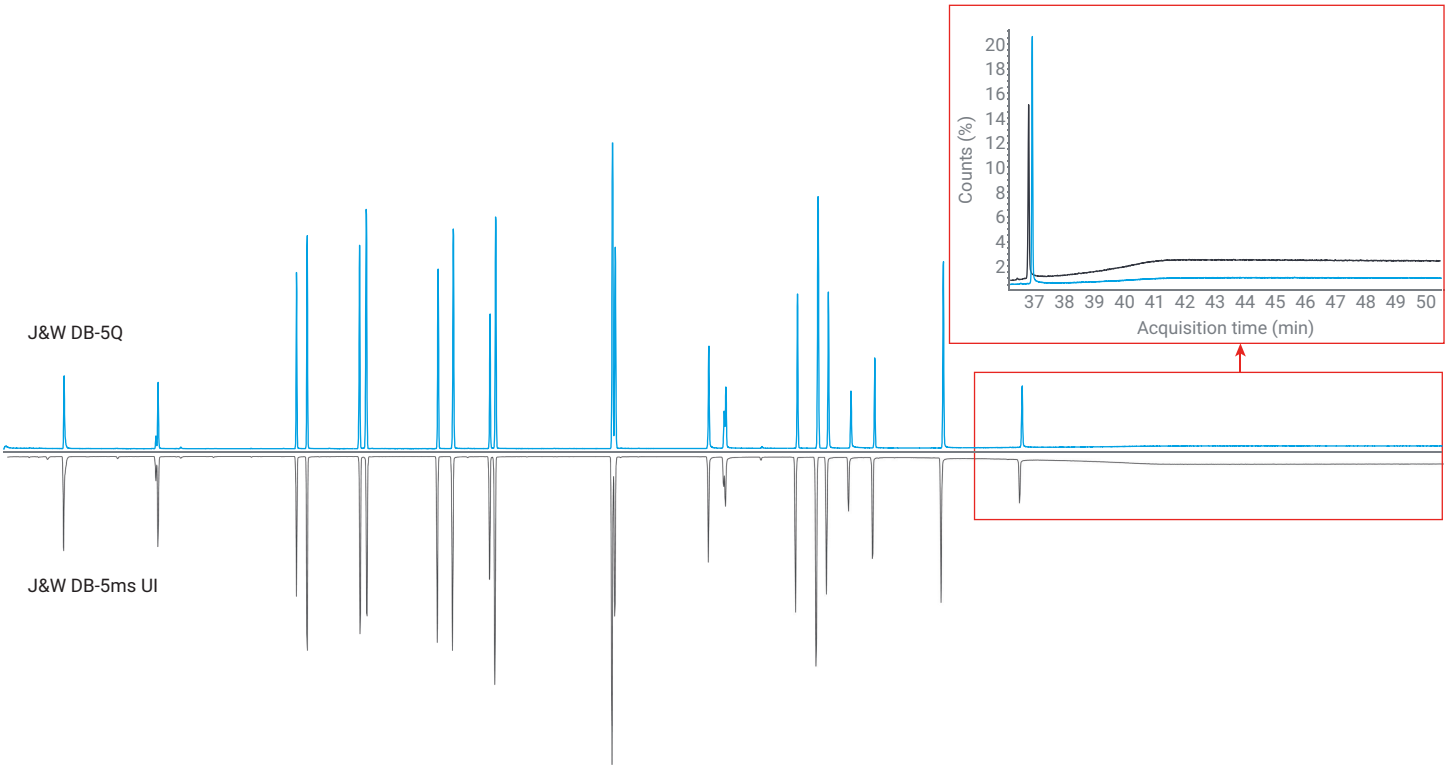

Figure 1. Agilent J&W DB-5Q column has similar selectivity to the J&W DB-5ms UI column, as demonstrated in the analysis of pesticides compounds, zoomed in on a comparison of the perceived bleed at the upper limit of the temperature program.

Phase durability and retention time stability

To stress the robustness of the GC system in a real-world application, a heavy soil matrix was diluted in DCM and analyzed over multiple thermal cycles. The inlet liner and septum were replaced every 20 matrix injections, as previous studies[5] indicated it necessary to maintain performance and properly protect the analytical phase. Figure 2 demonstrates that after 200 matrix injections, the retention times were consistent. In Table 5, the %RSD for the replicate injections of the pesticide standard over the 200 matrix injections were all less than 3%, and only two having a %RSD greater than 2.5% indicating the robustness of the DB-5Q column phase.

Utilizing Mass Hunter Unknowns Analysis software, the spectra for the pesticide compounds were deconvoluted and searched through NIST20. The library match factors displayed in Table 5 were all determined to be greater than 95, indicating a great level of accuracy and proper identification of pesticides even after 200 injections of a soil matrix.

Table 5. Retention time repeatability results over 200 soil matrix injections, analyzed on the Agilent J&W DB-5Q GC column.

Peak	Compound	Retention Time (min)	%RSD	NIST Match Factor
1	Dichlorvos	5.917	1.13%	97.25
2	Mevinphos	8.902	1.24%	97.98
3	Ethalfluralin	13.368	1.60%	97.67
4	Trifluralin	13.717	1.60%	95.69
5	Atrazine	15.412	2.14%	98.71
6	Lindane (γ-BHC)	15.604	1.35%	98.45
7	Chlorpyrifos-methyl	17.928	1.71%	98.40
8	Heptachlor	18.413	1.68%	97.89
9	Malathion	19.614	2.13%	98.28
10	Chlorpyrifos	19.797	1.85%	99.16
11	DDE, p,p'-	23.570	1.89%	98.59
12	Dieldrin	23.648	1.84%	96.13
13	Hexazinone	26.687	2.10%	96.00
14	Propargite	27.241	2.25%	91.82
15	Leptophos	29.547	2.05%	96.23
16	Mirex	30.198	2.01%	97.65
17	Fenarimol	30.546	2.12%	97.97
18	Coumaphos	32.053	2.36%	96.75
19	Etofenprox	34.271	2.85%	95.53
20	Deltamethrin	36.819	2.70%	96.97

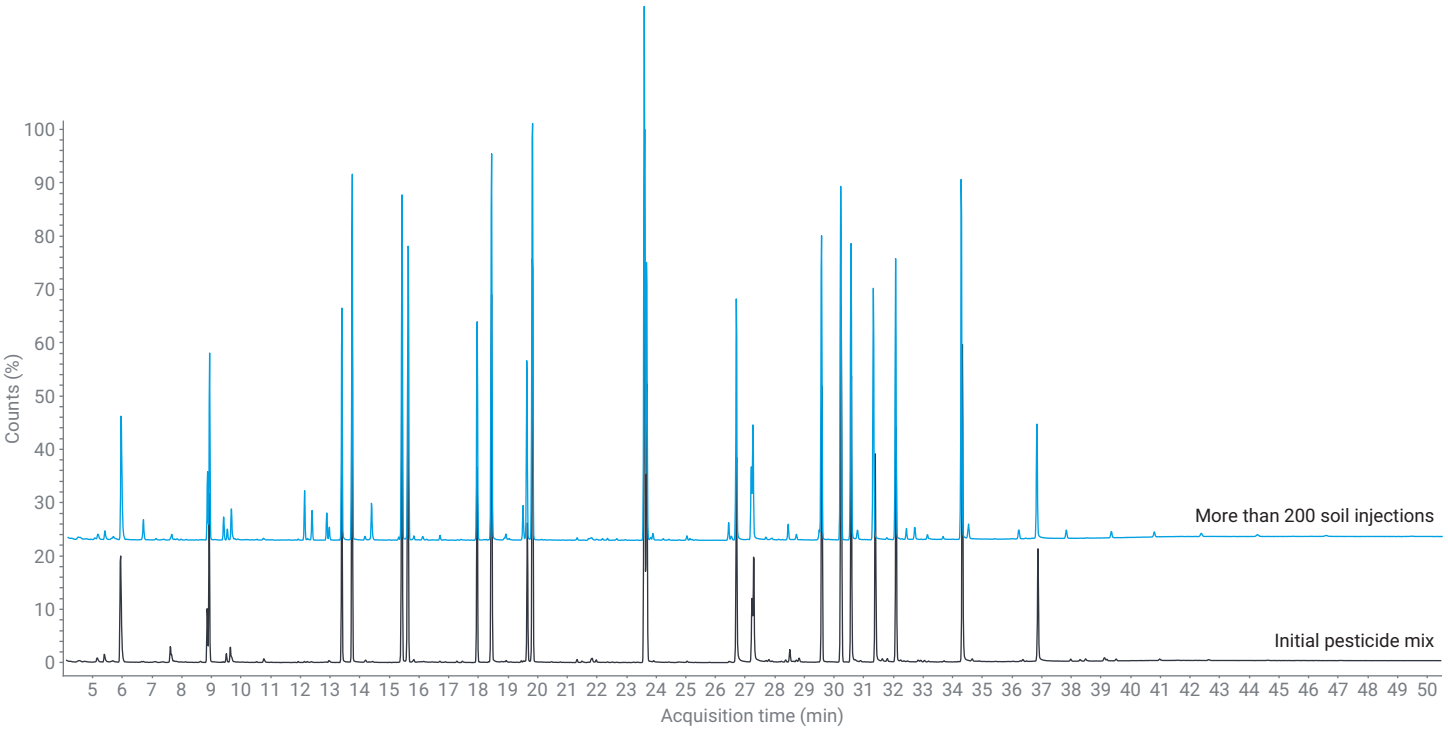

Figure 2. Pesticide checkout mix analyzed before and after 200 soil matrix injections on an Agilent J&W DB-5Q GC column.

Optimal sensitivity for the analysis of pesticides

Examining the sensitivity of the HES 2.0 ion source, calibration standards were prepared and analyzed on a DB-5Q column and acquired using dMRM. Figure 3 demonstrates the standard of 10 ppb pesticides and zooms in on two compounds, ethalfluralin and deltamethrin; their signal-to-noise ratios were calculated to be 232 and 362, respectively. The calibration of ethalfluralin is demonstrated in Figure 4 and shows an R^2 greater than 0.99 for the linear calibration over the range of 10 to 500 ppb allowing for precise quantitation over a wide dynamic range.

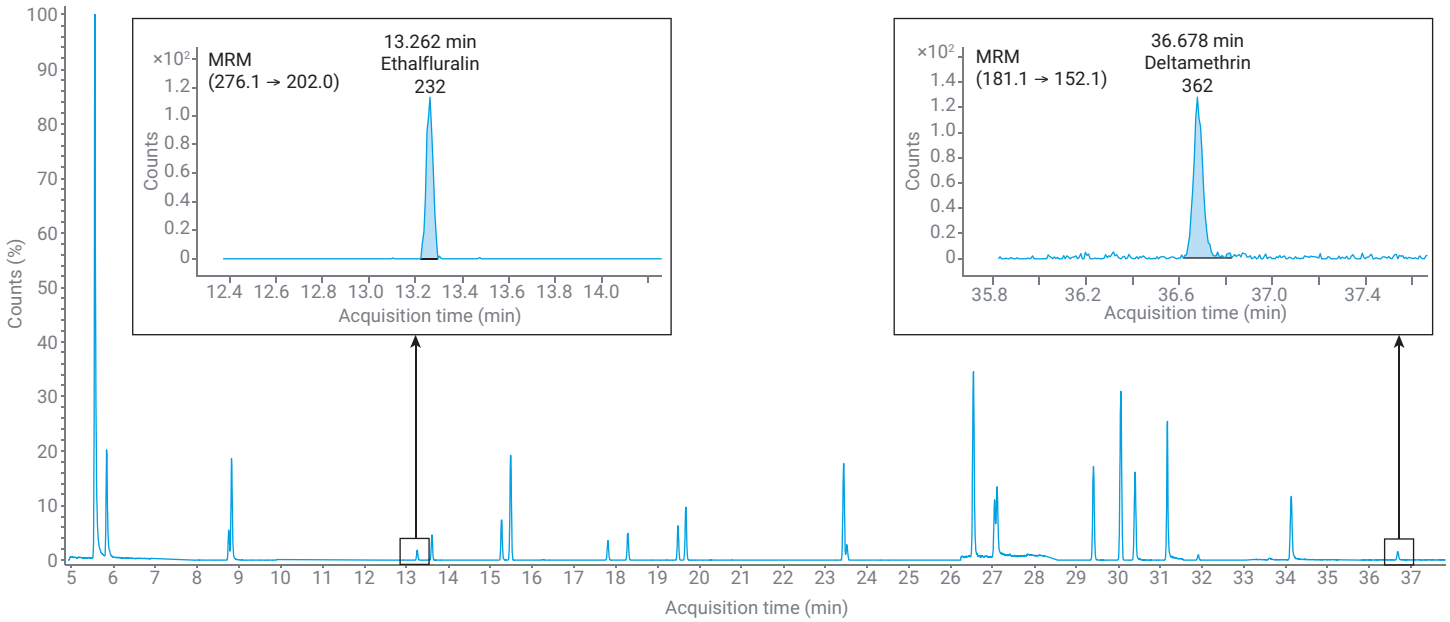

Figure 3. The 10 ppb calibration standard on the Agilent 7010D GC/MS.

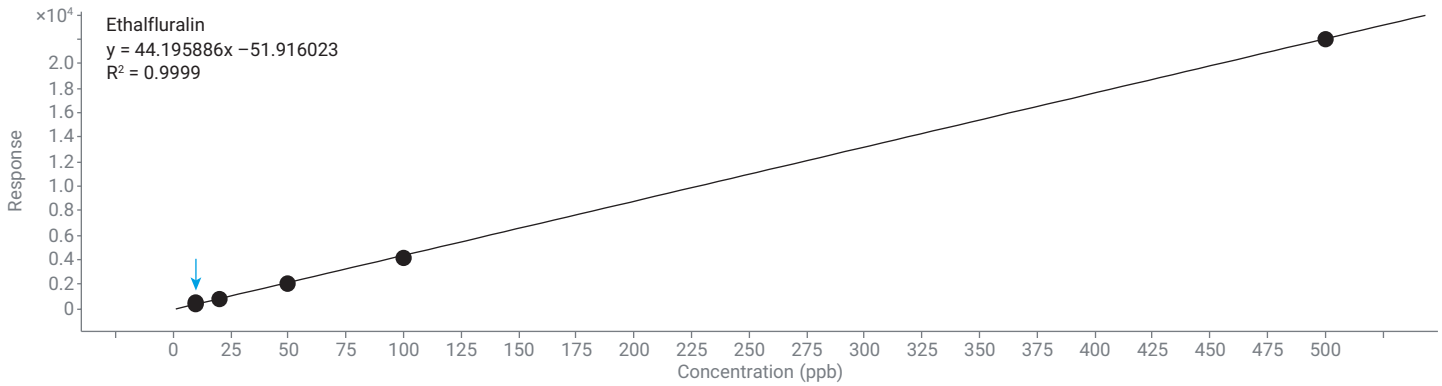

Figure 4. Calibration of ethalfluralin: range 10 to 500 ppb prepped in DCM.

Conclusion

This application note demonstrated that the Agilent J&W DB-5Q column has identical selectivity to the Agilent J&W DB-5ms UI column, allowing for seamless adoption and use with existing retention time locking methods. The robust DB-5Q column phase maintained retention time stability even when analyzing heavy matrices and temperature cycling. The increased thermal stability not only decreased the baseline at higher temperatures but also allowed for less spectral interference and high library match factor scores. The analytical performance of the Agilent J&W DB-5Q column coupled with the Agilent 7010D with the HES 2.0 ion source allows for optimal sensitivity and wide dynamic range of pesticide compounds.

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