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TITLE: HARNESSING THE FLEXIBILITY OF THE HALO® COLUMN TO INFORM THE DEVELOPMENT OF AUTOMATED TOXICOLOGY WORKFLOWS

MARKET SEGMENT: FORENSICS



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ABSTRACT

The global adoption of analytical automation accelerated significantly following the COVID-19 pandemic, shifting automation from a desirable option to a necessity within analytical laboratories. A critical step in this transition is the precise conversion of manual analytical methods into robust, automated workflows, necessitating stringent performance verification to ensure comparability and reliability.

This technical note explores the HALO 90 Å Biphenyl, 2 µm, 2.1 x 100 mm HPLC column, showcasing its versatility and performance advantages in automated analytical setups. Three distinct automated toxicology workflows were developed and evaluated, highlighting the column's flexibility and pivotal contribution to successful automation implementation.

INTRODUCTION

Analytical automation is rapidly transforming the landscape of analytical chemistry. While automation was once considered merely advantageous, the global pandemic underscored its essential role, shifting perceptions from "nice-to-have" to "need-to-have."

A pivotal challenge in adopting automation lies in accurately translating established manual workflows into their fully automated equivalents. Following the design and configuration of suitable automated hardware, rigorous analytical performance evaluation is crucial to confirm that automated processes consistently meet or exceed the performance of their manual counterparts.

Da Vinci Laboratory Solutions (DVLS) UK and Ireland specialises in designing and implementing tailored automation platforms. Within the DVLS Applications Demo Laboratory, each proposed automated workflow undergoes extensive development, optimization, and evaluation. Given the variety of applications assessed, any consumable integrated into these workflows must deliver exceptional flexibility, reproducibility, and versatility.

The HALO® Biphenyl stationary phase uniquely combines hydrophobic, aromatic, and polar selectivity, offering a versatile alternative to standard reversed-phase packing, especially beneficial for polar analytes or compounds susceptible to π-π interactions and mild steric effects.

This technical note highlights the robust performance and versatility of the HALO® Biphenyl column, illustrating its successful application within DVLS' Applications Demo Lab across three distinct automated toxicology workflows.

EXPERIMENTAL

An Agilent 1260 Infinity II HPLC, coupled to an Agilent 6470A triple quadrupole mass spectrometer (Santa Clara, CA) was used for the analysis of the prepared samples. The standards were obtained partially from Cerilliant (Round Rock, TX) and partially were provided by our collaborators. A HALO 90 Å Biphenyl (Advanced Materials Technology, Wilmington, DE) was chosen for the chromatographic separation. LC-MS grade solvents and modifiers were sourced from ROMIL (Cambridge, UK) and the matrix samples were provided by collaborators.

KEY WORDS:

drugs of abuse, drug metabolites, HALO 90 Å Biphenyl, 2 µm, 2.1 x 100 mm HPLC column, Analytical Automation, LC-MS

WORKFLOW 1 and 2– Drugs of Abuse in URINE and HAIR

LC CONDITIONS:
Column: HALO 90 Å Biphenyl, 2 µm, 2.1 x 100 mm
Part Number: 91812-611
Mobile Phase A: Water/0.1% Formic Acid
Mobile Phase B: Methanol/0.1% Formic Acid
Gradient: (Time - %B) 0 min – 5%, 0.5 min – 5%, 5.5 min – 98%, 12.0 min – 98%, 12.01 – 5%, 17.00 – 5%
Flow: 0.4 mL/min.
Temperature: 40 °C
Injection volume: 10 µL

MS SOURCE and ANALYSER CONDITIONS (ESI Positive)
Gas Temperature: 300 °C
Gas Flow: 10 L/min.
Nebuliser: 45 psi
Sheath Gas Temperature: 275 °C
Sheath Gas Flow: 12 L/min.
Capillary: 3500 V
Acquisition Mode: Dynamic MRM

Compound Name	Retention Time (min)	MRM Transition Q1>Q3	Fragmentor	CE [eV]
6-Acetyl codeine	5.33	342.2>165.1	110	40
6-Acetyl codeine d3	5.31	345.2>225.1	110	30
6-Acetyl morphine	4.57	328.2>165.1	110	44
6-Acetyl morphine d3	4.55	331.2>165.1	110	44
AEME	3.59	182.2>91.1	90	40
AEME d3	3.57	185.2>91.1	90	40
Alprazolam	8.04	309.2>281.2	130	28
Alprazolam d5	8.02	314.2>279.1	130	28
Amitriptyline	7.08	278.2>91.1	110	30
Amitriptyline d3	7.06	281.1>91.1	110	30
Amphetamine	3.94	136.1>119.1	50	4
Amphetamine d8	3.92	144.1>127.1	50	4
Benzoylecgonine	5.40	290.2>105	90	50
Benzoylecgonine d3	5.38	293.2>105.1	90	50
Buprenorphine	5.79	468.3>396.2	180	20
Buprenorphine d4	5.77	472.3>58.9	180	40
Citalopram	5.67	325.2>262.2	110	30
Citalopram d6	5.65	331.2>262.2	110	30
Chlordiazepoxide	7.00	300.1>227.1	100	30
Chlordiazepoxide d5	6.98	305.1>232.1	100	30
Chlorpheniramine	5.47	272.2>230.1	100	30
Chlorpheniramine d5	5.45	281.1>230.1	100	30
Clonazepam	7.60	316>270	120	28
Clonazepam d4	7.58	320.1>274.0	120	28
Cocaethylene	5.57	318.1>82	110	36
Cocaethylene d3	5.55	321.1>85.1	110	36
Cocaine	5.36	304.2>182.1	100	20
Cocaine d3	5.34	307.2>185.1	100	16
Codeine	5.16	300>215.1	110	40
Diacetylmorphine	6.10	370.2>268.2	110	30

Compound Name	Retention Time (min)	MRM Transition Q1>Q3	Fragmentor	CE [eV]
Diacetylmorphine d9	6.08	379.2>272.2	110	30
Diazepam	8.29	285.1>193.1	120	40
Diazepam d5	8.27	290.1>198.1	120	40
Dihydrocodeine	5.14	302.2>128.1	120	65
Dihydrocodeine d6	5.12	308.2>202.1	120	36
Diphenhydramine	6.66	256.2>167	120	30
Diphenhydramine d3	6.64	259.2>167	120	30
EDDP	7.03	278.2>234.1	140	36
EDDP d3	7.01	282.2>235.1	140	36
Fentanyl	6.74	337.3>105	100	44
Fentanyl d5	6.72	342.3>105	100	44
Flurazepam	6.83	388.2>315.1	110	24
Flurazepam d4	6.81	394.2>321.1	110	16
Gabapentin	4.49	172.2>137.0	70	16
Gabapentin d10	4.48	182.2>147.2	70	12
Ketamine	6.02	238.1>125.0	110	30
Ketamine d4	6.01	242.1>129.1	110	30
Lorazepam	7.86	321.0>275.0	100	20
m-Hydroxybenzoylecgonine	5.71	309.2>168.0	90	30
m-Hydroxybenzoylecgonine d3	5.70	309.2>171.0	90	30
MDA	4.92	180.1>105.1	60	8
MDA d5	4.91	185.1>168.1	60	8
MDMA	5.29	194.1>133.0	70	15
MDMA d5	5.28	199.2>165.0	70	8
MDPV	6.29	276.2>126.1	100	30
MDPV d8	6.28	284.2>134.1	100	30
Metamphetamine	4.93	150.2>119.1	60	10
Methamphetamine d5	4.92	155.2>121.1	60	10
Mephedrone	5.47	178.2>145.1	100	30
Mephedrone d3	5.46	181.2>148.1	100	30
Methadone	7.22	310.3>218.0	90	20
Methadone d3	7.21	313.3>220.0	80	20
Mirtazapine	6.36	266.2>208.9	100	30
Mirtazapine d3	6.35	269.2>208.9	100	30
Morphine	2.52	286.2>165.1	120	48
Morphine d6	2.51	292.2>165.1	120	48
Morphine 3-glucuronide	4.50	462.2>286.1	120	40
Morphine 6-glucuronide	4.50	462.2>201.0	120	30

Compound Name	Retention Time (min)	MRM Transition Q1>Q3	Fragmentor	CE [eV]
Norfentanyl	5.81	233.2>83.9	100	45
Norfentanyl d5	5.80	238.5>83.9	100	45
Norbuprenorphine	6.39	414.3>83.0	160	60
Norbuprenorphine d3	6.38	417.3>83.0	160	60
Norcocaine	6.27	290.2>136.1	110	30
Norcocaine d3	6.26	293.2>136.1	110	30
Nordiazepam	7.82	271.1>140.0	110	40
Nordiazepam d5	7.81	276.1>140.0	110	40
Norketamine	5.80	224.1>125.1	70	25
Norketamine d4	5.79	228.1>129.1	70	25
Nortriptyline	7.06	264.2>233.1	100	30
Nortriptyline d3	7.05	267.1>233.2	100	30
O-desmethyl tramadol	5.19	250.2>58.0	70	50
O-desmethyl tramadol d6	5.18	256.3>64.0	70	50
Oxazepam	7.59	287.1>241.1	100	25
Oxazepam d5	7.58	292.1>246.1	100	25
Oxycodone	5.32	316.2>241.2	100	30
Oxycodone d3	5.31	319.2>244.2	100	30
Oxymorphone	3.10	302.2>227.1	100	30
Oxymorphone d3	3.09	305.2>230.1	100	30
Pregabalin	3.33	160.1>96.9	60	40
Pregabalin d6	3.32	166.1>148.0	60	25
Propranolol	6.52	260.1>116.1	100	30
Propranolol d7	6.51	267.2>116.1	100	30
Promethazine	6.95	285.2>85.9	100	30
Promethazine d3	6.94	288.2>88.9	100	30
Temazepam	8.01	301.1>255.1	100	24
Temazepam d5	8.00	306.1>260.1	100	24
Tramadol	5.95	264.2>58.0	70	16
Tramadol d3	5.94	268.2>58.0	70	16
Zopiclone	6.18	389.2>245.1	60	16
Zopiclone d4	6.17	393.2>245.1	60	16
Zolpidem	6.61	308.2>235.2	130	40
Zolpidem d6	6.60	314.2>235.2	130	40

Table 1: Retention times and MRM transitions for the selection of drugs of abuse and metabolites analysed in workflows 1 and 2

WORKFLOW 3 - Cannabinoids and Metabolites in Mitra devices (URINE)

LC CONDITIONS:
Column: HALO 90 Å Biphenyl, 2 µm, 2.1 x 100 mm
Part Number: 91812-611
Mobile Phase A: Water 0.1% Formic Acid
Mobile Phase B: Methanol 0.1% Formic Acid
Gradient: (Time - %B) 0 min – 30%, 2.0 min – 82.5%, 4.0 min– 98%, 10.0 min – 98%, 10.01 – 5%, 15.00 – 5%
Temperature: 50 °C
Injection Volume: 10 µL

MS SOURCE and ANALYSER CONDITIONS (ESI Positive)
Gas Temperature: 300 °C
Gas Flow: 10 L/min
Nebuliser: 45 psi
Sheath Gas Temperature: 375 °C
Sheath Gas Flow: 12 L/min.
Capillary: 3500 V
Acquisition Mode: Dynamic MRM

Compound Name	Retention Time (min)	MRM Transition Q1>Q3	Fragmentor	CE [eV]
11-COOH-CBN	6.11	341.2>323.4	110	30
6-OH CBD	5.33	331.3>271.2	110	25
Cannabidiol (CBD)	6.05	315.2>193.2	110	24
CBD Glucuronide	5.49	491.3>315.2	110	30
Cannabinol (CBN)	6.38	311.2>223.2	110	24
THC	6.50	315.3>193.1	110	24
THC D3	6.48	318.3>196.0	110	24
THC-COOH	6.14	345.2>299.1	110	32

Table 2: Retention times and MRM transitions for the selection of drugs of abuse and metabolites analysed in workflow 3

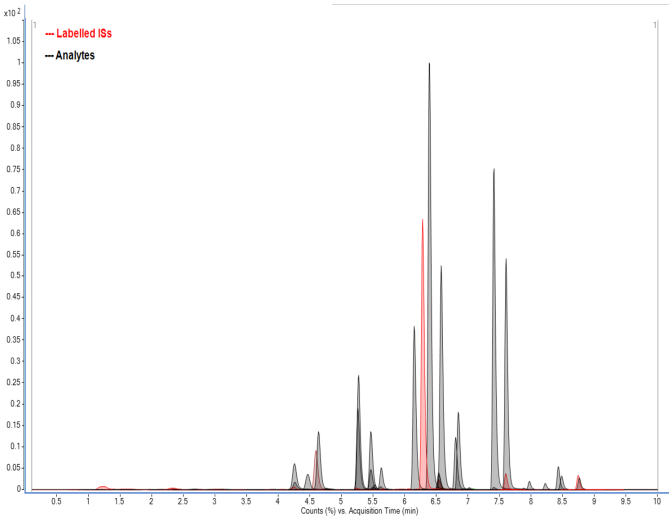


Figure 1: Optimized chromatographic method for the separation of the selection of drugs of abuse in workflow 1 (extracted ion chromatogram for quantifier transitions)

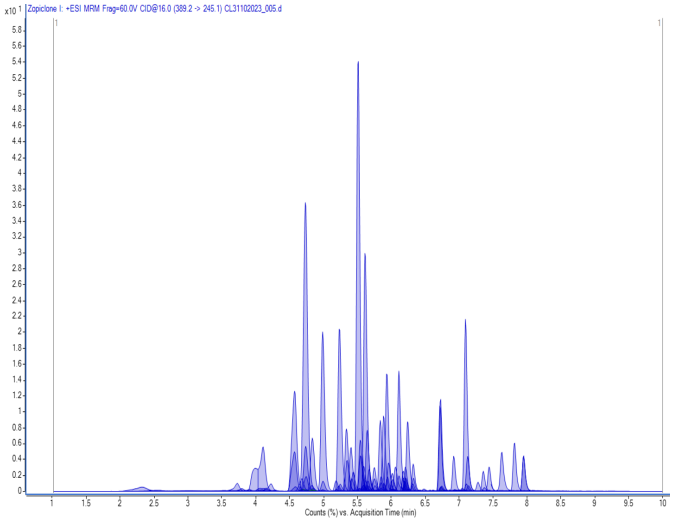


Figure 2: Optimized chromatographic method for the separation of the selection of drugs of abuse in workflow 2 (extracted ion chromatogram for quantifier transitions)

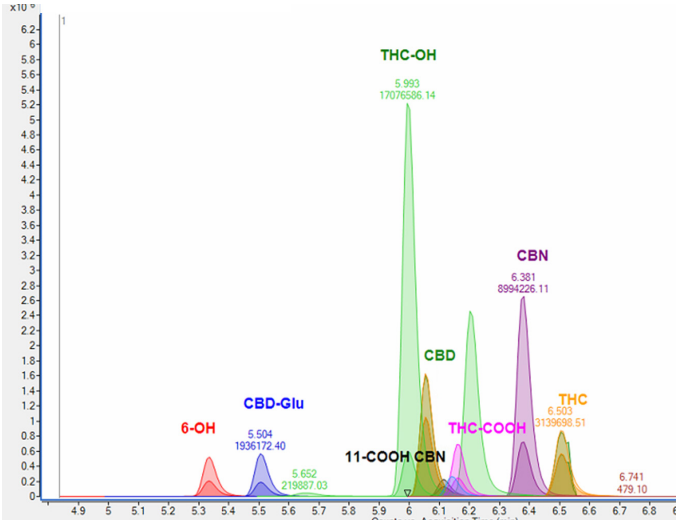


Figure 3: Optimized chromatographic method for the separation of the selection of drugs of abuse in workflow 3 (extracted ion chromatogram for quantifier transitions)

RESULTS

Each project included a preliminary development phase of the automation workflow, followed by the final sample analysis employing the optimized automated sample preparation to evaluate its analytical performance.

For workflows 1 and 2, sample analysis required acquiring three batches over three separate days to assess the reproducibility of the developed assay. Each batch comprised procedural blanks, a six-point matrix-matched calibration curve, bracketing QC at low and high concentration ranges, and a varied number of matrix samples (n ≥ 6). For workflow 3, only the preliminary development phase was carried out, with sample analysis to be performed at a later stage.

Matrix-matched standards for quantification and relevant matrix samples were analysed using the optimized chromatographic separation on the HALO 90 Å Biphenyl.

Linearity for workflow 1 (DoA in urine) ranged from 0.9944 to 1.000 (concentration range 1-100 ng/mL), while linearity for workflow 2 (DoA in hair) ranged from 0.9971 to 0.9999 (concentration range 20-400 ng/mL). Inter-day precision as % RSD for calculated concentrations for the matrix-matched calibrants across the entire investigated range was below 20% for all analytes, with the majority being below 10%.

Figure 1, 2 and 3 show the extraction ion chromatograms (quantifier ions) for the optimized chromatography in matrix-matched standard (100 ng/mL) for workflow 1, 2 and 3 respectively.

Separation of the target analytes for the three matrices was successfully achieved within 10 minutes runtime, with most peaks revealing a symmetric and sharp peak shape.

Each project was carried out over a two- to three-week period, with injection numbers of 224 for workflow 1, 98 for workflow 2, and 56 for workflow 3. Retention time precision, expressed as RSD%, for the entire range of analytes across each project, ranged between 0.1% and 1.3%.

We would like to thank Camilla Liscio, PhD – Principal Scientist, DVLS UK and Ireland and Colin Hastie, PhD- Application Chemist, DVLS UK and Ireland for their evaluation of the HALO 90 Å Biphenyl, 2 µm, 2.1 x 100 mm HPLC column and for sharing their experimental results and technical report for publication.

CONCLUSION:

The implementation of analytical automation in the laboratory relies on accurately translating the original manual workflow into its automated equivalent. To support this process and guarantee a robust and reliable automated solution, a thorough evaluation of the analytical performances of the newly developed automated workflow needs to be carried out. The DVLS Applications Demo Laboratory specialises in the development, optimization, and evaluation of automated workflows for various sectors. Given the variety of applications assessed, any consumable integrated into these workflows must deliver exceptional flexibility, reproducibility, and versatility.

The HALO® Biphenyl column was used across three projects focused on developing distinct automated toxicology workflows. The results showcased the high adaptability of this column in combination with its high standard chromatographic performance, in terms of separation efficiency, peak symmetry and retention time consistency.

These examples demonstrated not only the column's inherent adaptability but also its pivotal role in seamlessly translating manual analytical processes into fully automated workflows.

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