Application Note

Forensics

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Using a Deans Switch to Divert Derivatizing Agents from the EI Source for Improved Robustness in High-Throughput Anti-Doping GC/MS/MS Workflow

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Abstract

Derivatization reagents such as MSTFA are known to foul electron ionization sources, driving frequent cleanings and retunes in high-throughput anti-doping workflows. This application note demonstrates the use of a Deans switch in the analysis of anabolic steroids in urine samples, as well as for anti-doping control workflows. A Deans switch was used to vent solvent and early eluting derivatization agents away from the mass spectrometer and into a restrictor vent during the solvent vent window. Following this, the analytical column effluent was returned to the MS for quantitation. On an Agilent 7000 Series GC/TQ operated with hydrogen carrier gas, cleaning intervals increased from approximately 350–400 to ≥ 1,500 injections, while maintaining required sensitivity and stable peak shapes for steroids. On an Agilent 7010 Series GC/TQ operated with helium carrier gas, cleaning frequency improved from approximately every 300 to 350 injections to approximately 900 injections before intervention. With routine batches of 80 samples, source cleaning shifted from weekly to every 2 to 3 weeks. This approach, which preserves data quality while reducing maintenance and increasing uptime, integrates seamlessly with validated anti-doping sample preparation and acquisition methods.

Introduction

N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) is widely used to form trimethylsilyl (TMS) derivatives of steroids and their nonhydrolyzed sulfate metabolites for GC/MS(/MS) analysis. However, when introduced into the electron ionization (EI) source, MSTFA and its byproducts can deposit SiO_2-like insulating layers, forcing rapid increases in ion body and repeller voltages and necessitating abrasive cleaning—especially in high-throughput labs. Importantly, this SiO_2-like insulating layer can be formed even when the filament is turned off (for example, during the solvent-vent period), so simply extending the solvent-vent time until the MSTFA peak has passed does not fully mitigate source fouling. Using a Deans switch to divert unreacted derivatization reagent during the solvent vent window reduces source exposure without altering established analytical selectivity. A Deans switch (Figure 1) is an in-oven capillary flow device that enables precise, programmable redirection of column effluent between multiple outlets—such as to a detector or to vent—by electronically controlling flow paths during a GC run.[1] This technology allows analysts to selectively divert unwanted components, like solvent or derivatization byproducts, away from the mass spectrometer, protecting sensitive detector surfaces and improving method robustness.

The described GC/TQ systems and Deans switch configurations are optimized for the targeted analysis of a comprehensive panel of anabolic steroids and their metabolites in urine matrices. These include both endogenous steroids—such as testosterone, dihydrotestosterone, dehydroepiandrosterone, and their respective conjugates—and exogenous compounds that may indicate the use of banned substances.

In this work, an Agilent Deans switch was introduced into two routine anti-doping workflows to address the persistent issue of EI source contamination associated with the derivatizing agents and column bleed, as demonstrated in two representative applications:

- Confirmation and quantitation of endogenous steroids in urine with the Agilent 7000 Series GC/TQ using hydrogen carrier gas
- Routine screening of exogenous steroids and other doping agents in complex/dirty samples with the Agilent 7010 Series GC/TQ using helium carrier gas

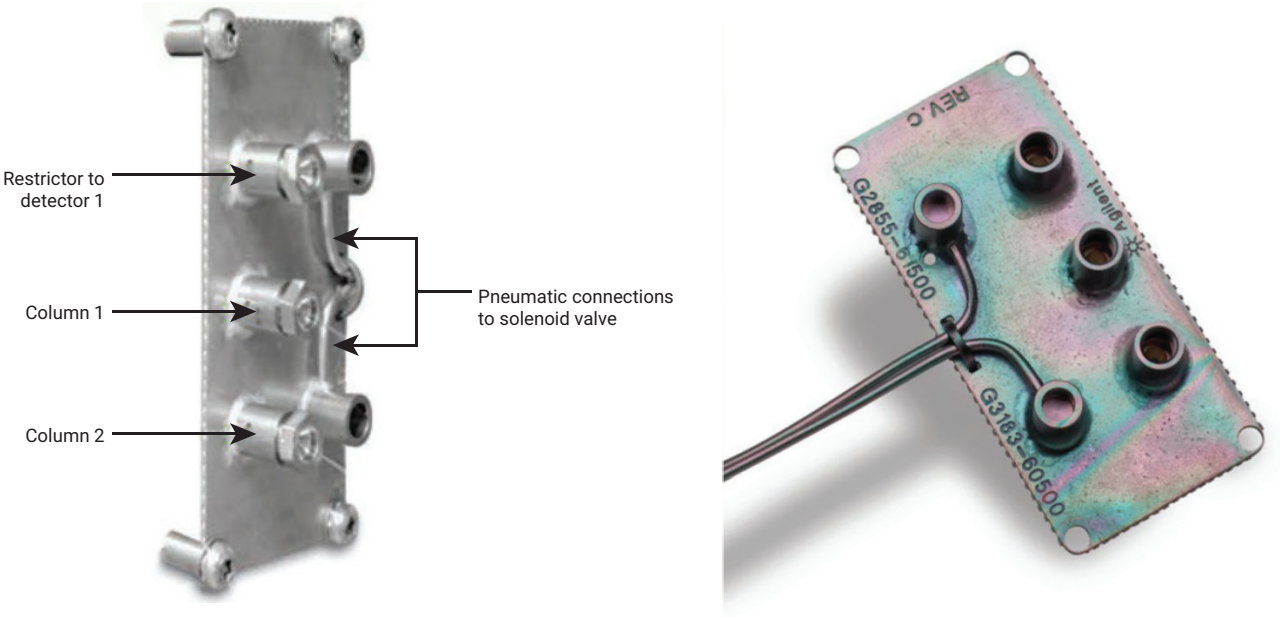


Figure 1. Photo of Agilent capillary flow technology Deans switch.

Experimental

Samples

The analysis of anabolic steroids in urine for anti-doping control requires robust sample preparation workflows that can efficiently handle complex biological matrices and deliver reliable results across a wide range of target compounds. In this study, two complementary approaches were employed to address the needs of routine screening and confirmation: one designed for high-throughput quantitation of endogenous steroids, and another optimized for high-sensitivity analysis in challenging, high-matrix samples. Both workflows incorporate solid phase extraction (SPE) and derivatization strategies tailored to maximize recovery, selectivity, and analytical performance, ensuring compliance with regulatory standards and supporting the rigorous demands of anti-doping laboratories.

7000 Series GC/TQ with hydrogen carrier gas

For confirmation and quantitation of endogenous steroids, urine samples were prepared following a validated protocol optimized for hydrogen carrier gas workflows.[2] After sample collection, each aliquot was subjected to hydrolysis and automated SPE to concentrate the target analytes and minimize matrix effects. The dried extracts were derivatized using MSTFA:NH_4I:ethanethiol (500:4:2 v/v/v), ensuring efficient conversion of the steroids to their TMS derivatives.

Calibration curves were constructed using matrix-matched standards spiked with deuterated internal standards (ISTDs) to account for variability in extraction and derivatization efficiency. The prepared samples were injected in splitless mode to maximize sensitivity, and each batch included a calibration curve, system suitability tests, and QC samples to monitor method performance. This workflow supports robust, high-throughput quantification of a broad panel of endogenous steroids, meeting the sensitivity and selectivity requirements for anti-doping control.

7010 Series GC/TQ with helium carrier gas

Urine samples for anti-doping screening were collected and processed using a semi-automated workflow designed for high-throughput analysis. Further details on the sample preparation workflow can be found in a previous Agilent application note (5994-6702EN).[3] SPE was performed using Agilent Bond Elut cartridges to isolate the prohibited substances and remove matrix interferences. The extracts were then evaporated and derivatized with a mixture of MSTFA:NH_4I:ethanethiol (typically 500:4:2 v/v/v), which converts all doping agents into volatile TMS derivatives suitable for GC/MS/MS analysis. The final derivatized samples were transferred to GC vials and loaded onto the autosampler for injection. Each analytical batch included calibration standards, system blanks, and quality control samples to ensure accuracy and reproducibility.

GC/TQ instrumentation and Deans switch configuration

The analysis was performed using Agilent 7000 Series and 7010 Series triple quadrupole GC/MS (GC/TQ) systems. The 7000 Series GC/TQ was operated with hydrogen as the carrier gas, while the 7010 Series GC/TQ was operated with helium. Both systems were equipped with a Deans switch (Figure 2), which enabled selective venting of solvent and derivatization agents during the initial phase of the chromatographic run, protecting the EI source from contamination and extending maintenance intervals. When using a Deans switch, restrictors play a key role as they are required to maintain equal pressure across the detector channels. In this work, appropriate restrictor dimensions were determined using the Capillary Flow Technology (CFT) Calculator available for download at the GC Calculators and Method Translator Software page.[4]

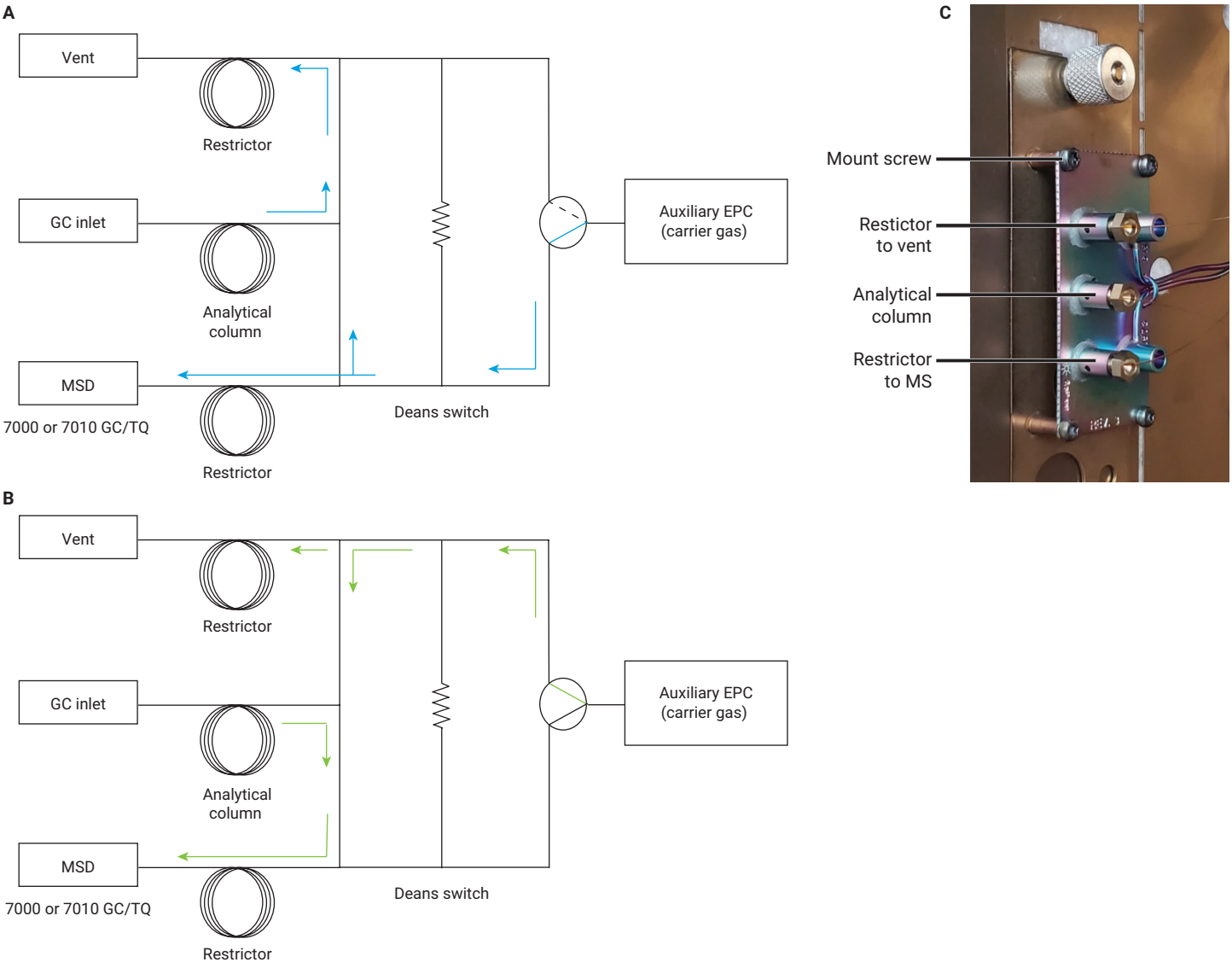
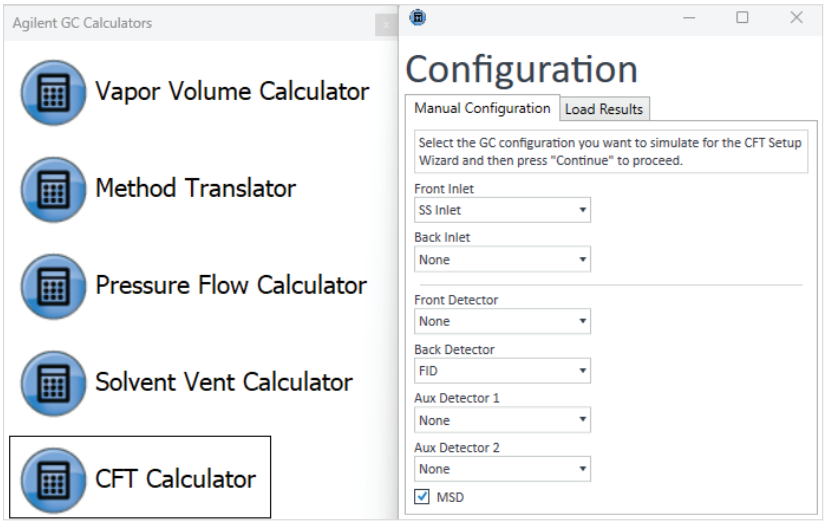


Figure 2. (A, B) Instrument configurations and (C) Deans switch plumbing in the GC oven.

The novel GC Capillary Flow Technology Wizard (Figure 3) allows for streamlined Deans switch setup, including calculating the length of the restrictors.



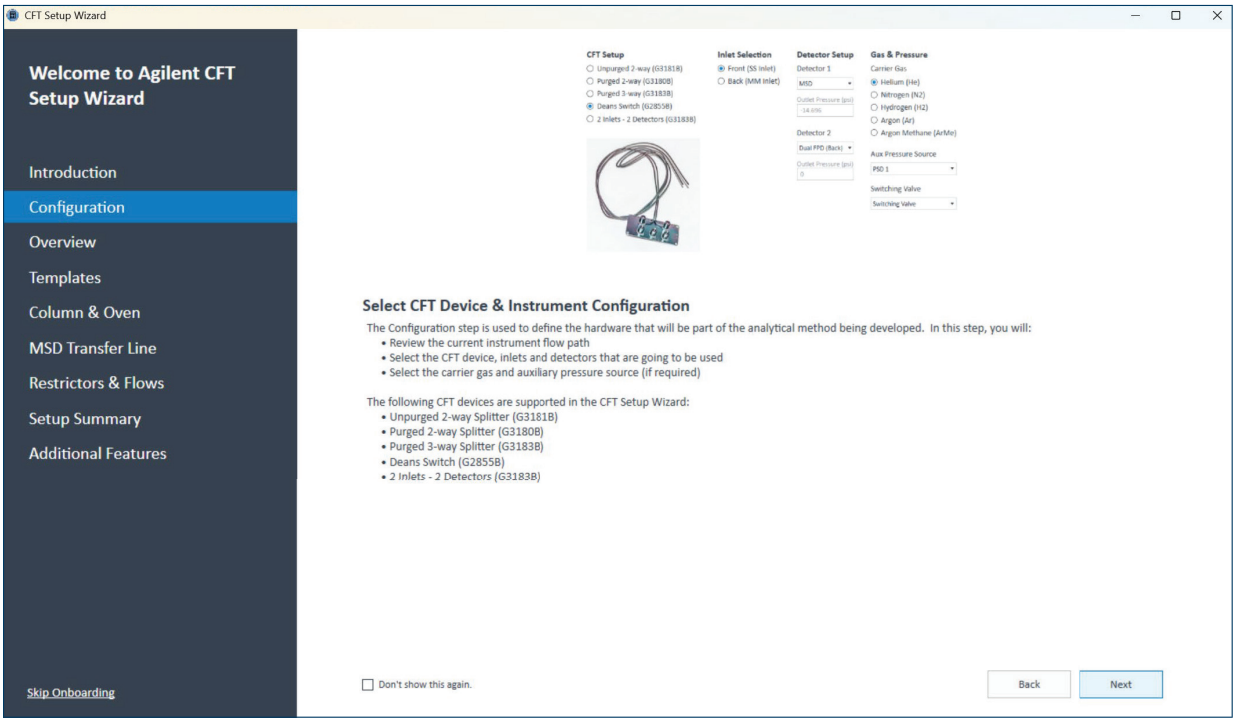


Figure 3. The Agilent CFT Setup Wizard and the supported CFT configurations.

Instrument parameters were optimized for forensic toxicological analysis of anabolic steroids and related metabolites in urine, with method details summarized in Table 1.

For hydrogen carrier gas workflows, additional best practices and conversion guidelines are available in the Agilent EI GC/MS Instrument Helium to Hydrogen Carrier Gas Conversion User Guide.[5]

Data acquisition and processing were performed using Agilent MassHunter acquisition software for GC/MS systems (version 10.2) and Agilent MassHunter Quantitative Analysis software (version 10.2).

Method validation

To assess external bias, 27 matrix-matched urine samples from the World Anti-Doping Agency (WADA) external quality assessment scheme (EQAS) were also analyzed, and z-scores for six compounds were calculated.[6]

Table 1. GC and MS conditions for forensic toxicological analysis.

Parameter	Agilent 7000 Series GC/TQ with Hydrogen Carrier Gas	Agilent 7010 Series GC/TQ with Helium Carrier Gas
Inlet	Split/splitless inlet (SSL)	Programmable temperature vaporizer inlet (PTV)
Mode	Splitless	Solvent vent
Purge Flow to Split Vent	50 mL/min at 0.75 min	50 mL/min at 0.75 min
Injection Volume	1.5 µL	3 µL
Inlet Temperature	280 °C	135 °C (0.1 min hold) 720 °C/min to 380 °C (1 min hold) 720 °C/min to 450 °C (1 min hold) 50 °C/min to 140 °C (0 min hold)
Inlet Liner	Agilent inlet liner, 4 mm, Ultra Inert (UI), single taper with glass wool (p/n 5190-2293)	Agilent inlet, PTV, liner, 1.8 mm, multibaffle, deactivated (p/n 5183-2037)
Restrictor (Deans Switch to Vent)	Agilent Ultimate Plus deactivated fused silica 0.91 m × 0.15 mm, 0 µm (p/n CP801510)	Agilent Ultimate Plus deactivated fused silica, 0.38 m × 0.18 mm, 0 µm (p/n CP801810)
Restrictor (Deans Switch to MS)	Agilent Ultimate Plus deactivated fused silica 0.85 m × 0.12 mm, 0 µm (p/n CP801206)	Agilent Ultimate Plus deactivated fused silica, 1.01 m × 0.12 mm, 0 µm (p/n CP801206)
Analytical Column	Agilent J&W Ultra 1 GC column 12 m × 200 µm, 0.11 µm (p/n 19091A-002)	Agilent J&W DB-35ms UI GC column, 15 m × 250 µm, 0.25 µm (p/n 122-3812UI)
Switching Time	4.9 min from vent to MS	1.5 min from vent to MS
Column Temperature Program (°C)	120 °C (0.15 min hold) 75 °C/min to 170 °C (0 min hold) 40 °C/min to 185 °C (0 min hold) 2.5 °C/min to 199 °C (0 min hold) 10 °C/min to 213 °C (0 min hold) 60 °C/min to 300 °C (0 min hold) Run time: 9.64 min	110 °C (0.1 min hold) 50 °C/min to 250 °C (0 min hold) 5 °C/min to 258 °C (0 min hold) 10 °C/min to 295 °C (0 min hold) 65 °C/min to 345 °C (2.2 min hold) 25 °C/min to 360 °C (0 min hold) Run time: 11.77 min
Carrier Gas and Flow Rate	Hydrogen, 1 mL/min constant flow	Helium, 0.8 mL/min (9 min hold) 0.5 mL/min to 1.2 mL/min (0 min hold) 0.5 mL/min to 2 mL/min (0 min hold)
Transfer Line Temperature	300 °C	310 °C
Triple Quadrupole Mass Spectrometer	Agilent 7000 Series GC/TQ system with extractor EI source	Agilent 7010 Series GC/TQ system with high-efficiency source (HES)
Electron Energy	70 eV	70 eV
Quench Gas (Helium)	2.25 mL/min **Note:** quench gas can be turned off and capped off when using hydrogen as a carrier gas	2.25 mL/min
Collision Gas (Nitrogen)	1.5 mL/min	1.5 mL/min
Ion Source Temperature	280 °C	280 °C
Quadrupole Temperature	150 °C	150 °C
Mode	Time segment multiple reaction monitoring (TS MRM), 80 MRMs across 4 time segments	Dynamic multiple reaction monitoring (dMRM), 490 MRMs
Tune	Etune: atunes.eiex.tune.xml	HES tune: atunes.eihs.jtune.xml

Results and discussion

Maintenance interval and analytical robustness

The implementation of the Deans switch in high-throughput anti-doping GC/MS/MS workflows yielded a substantial improvement in instrument maintenance intervals and overall analytical robustness. Both the 7000 and 7010 Series GC/TQ systems demonstrated significantly extended operation between source cleanings, with the limiting factor shifting from EI source fouling to column performance. Routine tuning and liner changes were performed as needed, but the overall stability of tuning parameters and sensitivity was maintained throughout extended sequences, supporting the method's suitability for routine anti-doping analysis.

Additionally, the use of a Deans switch allowed for changing columns without the need to vent the MS system. As a result, the source stays under vacuum conditions and does not get exposed to the atmosphere and moisture from the air. This substantially reduces the time that is otherwise required to cool the system before venting and to equilibrate the source after venting and starting up again. As a result, the time savings are doubled.

Evidence of Deans switch effectiveness: Baseline contaminant reduction

A critical benefit of the Deans switch is its ability to dramatically reduce the contaminant load entering the EI source during the solvent window. This effect is clearly illustrated in the total ion chromatograms (TICs) obtained from splitless injections of a dirty urine sample in scan mode. If no Deans switch is used, the baseline in the early part of the chromatogram is elevated, reflecting substantial contaminant influx to the source (Figure 4A). In contrast, when the Deans switch is employed and the flow is diverted to vent for the first 1.5 minutes, the early baseline is markedly lower, indicating that most solvent and derivatization byproducts are effectively removed before reaching the mass spectrometer (Figure 4B).

This reduction in baseline contamination translates directly to improved source longevity, reduced maintenance frequency, and more stable analytical performance, especially when processing complex, high-matrix samples typical of anti-doping workflows.

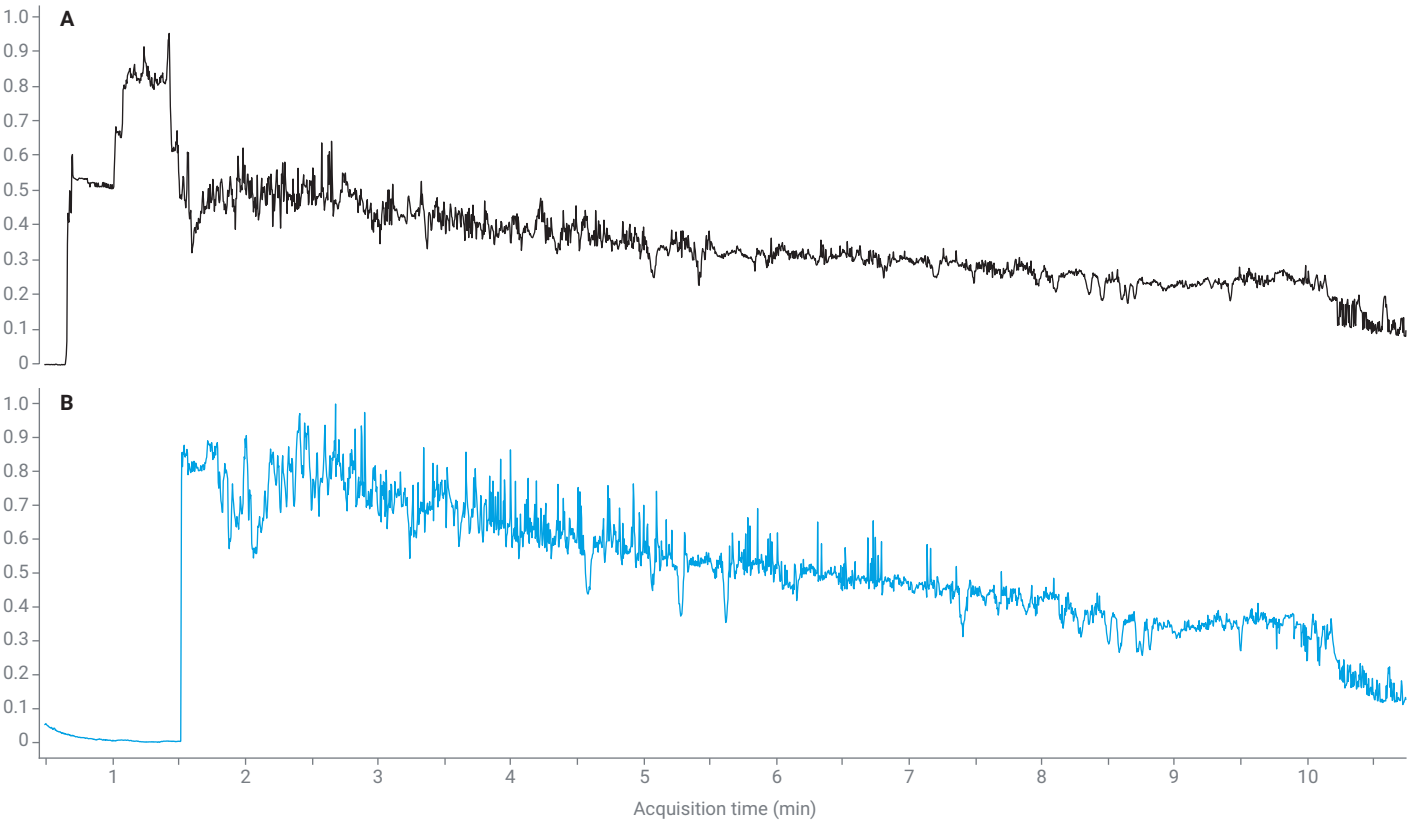


Figure 4. (A) 2 µL splitless injection of a dirty sample. Solvent delay: 0.2 minutes. No Deans switch; (B) 2 µL splitless injection of a dirty sample. Solvent delay: 0.2 minutes. Deans switch: 1.5 minutes.

Instrument performance with Deans switch

7000 Series GC/TQ with hydrogen carrier gas: For the 7000 Series GC/TQ system operating with hydrogen carrier gas, the Deans switch enabled extended operation without the need for frequent source cleaning. Intermediate manual tuning was performed approximately every 150 to 200 injections, yet tuning parameters remained remarkably stable. The ion body voltage increased only modestly—from 31 at the start to 38 after 1,000 injections, and up to 47 after 1,750 injections—while other parameters showed minimal drift. The liner was replaced after approximately 900 injections, coinciding with the onset of column performance decline, but the EI source maintained sensitivity and chromatographic integrity throughout. In total, the system achieved up to 2,000 injections before source cleaning was required, representing a five-fold increase in maintenance interval compared to conventional workflows without the Deans switch.

Figure 5 shows chromatograms of quantifier MRMs for five targets—androsterone, 5b-AND-3,17-dion, 5b-AND-3a,17b-diol, testosterone, and 6-OH-androstenedion—acquired at injections 50 and 1,065 within a 1,065-injection sequence.

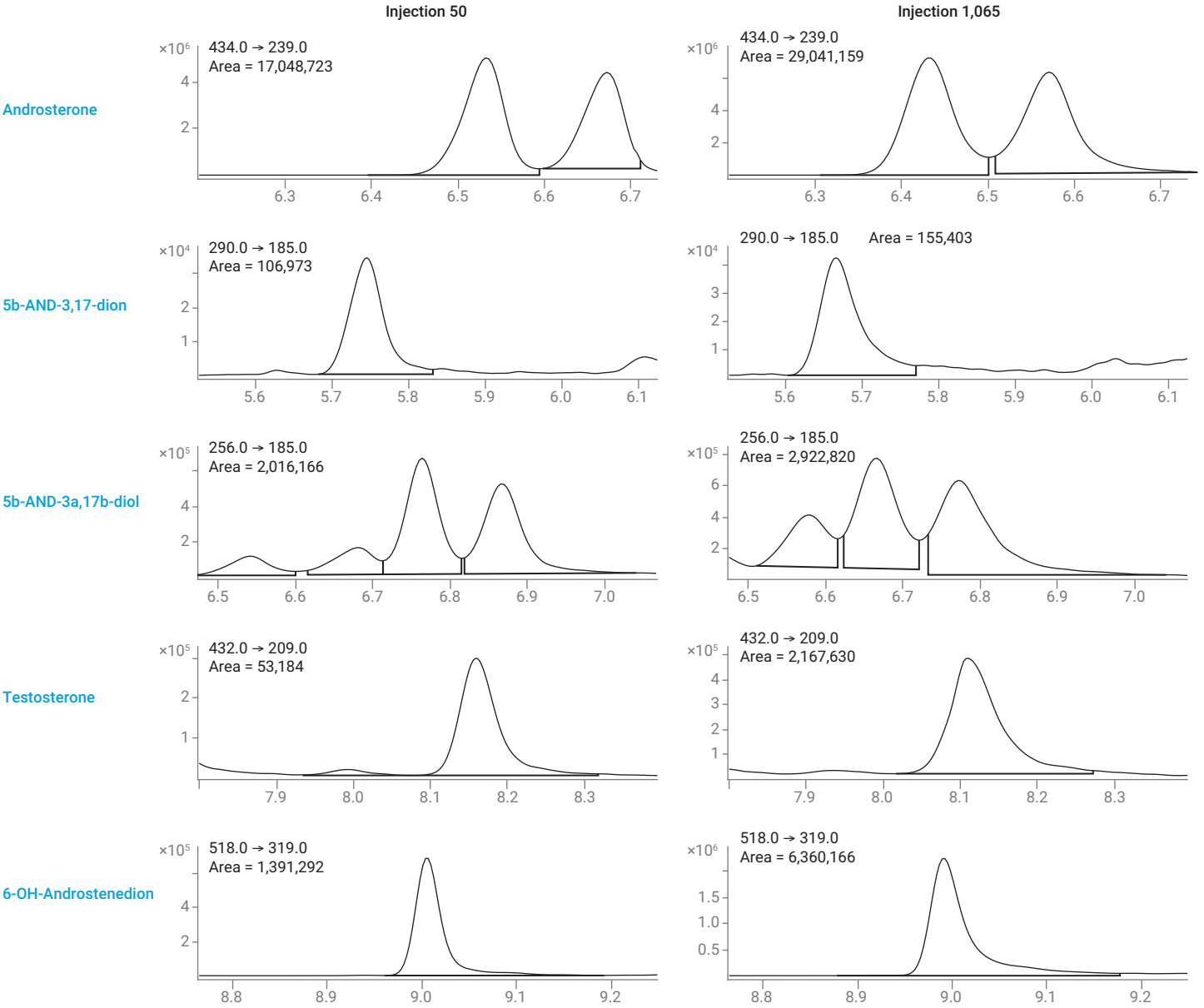


Figure 5. MRM chromatograms for five targets: androsterone, 5b-AND-3,17-dion, 5b-AND-3a,17b-diol, testosterone, and 6-OH-androstenedion. Data were acquired at injections 50 and 1,065.

These results demonstrate response stability and minimal retention time drift over the course of approximately 1,000 injections. It is of note that a slight deterioration in the peak shape at injection 1,065 was due to column aging, not source contamination. Column replacement typically occurs every 1,000 injections. The chromatograms in Figure 5 were acquired using the same column that was used for the entire duration of the experiment. The GC/TQ was tuned every 200 injections, followed by the ion ratio update for quantitation resulting in variation in area counts. Deuterated internal standards (ISTDs) were used to account for response variability.

Visual inspection of the source after extended use revealed a blue/gray coloration of the metal, in contrast to the typical brown/black deposits, further indicating reduced fouling. The method was then validated and transitioned into routine use, confirming its suitability for ongoing anti-doping applications. Notably, the same reference material was injected up to 1,750 times with maintained sensitivity, and a short validation after reinstalling the Deans switch with the old source showed excellent performance. These findings suggest that, with intermediate tuning and routine liner changes, the Deans switch approach can reliably extend source lifetime from 350–400 injections to approximately 2,000 injections.

7010 Series GC/TQ with helium carrier gas: For the 7010 Series GC/TQ system with helium carrier gas, similar benefits were observed. Prior to the introduction of the Deans switch, source cleaning was required every 300 to 350 injections, typically on a weekly basis for routine batches of 80 samples. After implementing the Deans switch, the number of injections between cleanings increased to approximately 900, with the limiting factor shifting from source fouling to column degradation. This translated to a cleaning frequency of every 2 to 3 weeks, significantly reducing instrument downtime and maintenance burden.

Chromatographic evaluation revealed that retention times shifted slightly later due to the altered flow path, but this was readily compensated by adjusting oven ramp rates, preserving method runtime and analytical performance. Peak shapes and sensitivity for key steroid targets remained consistent across a broad panel of analytes (Figure 6A). The ability to vent solvent and derivatization byproducts during the initial phase of the run proved critical for maintaining source cleanliness and analytical stability, especially when processing complex, high-matrix urine samples.

Notably, in the configuration using a Deans switch with the 7010 GC/TQ, a ramped column flow program was used to balance system performance and analytical runtime to maintain throughput. During the vent phase, a high flow was used to ensure there was enough pressure on the switch valve to operate, which also enabled faster analysis. During this vent time, no flow went to the MS source as analysis was not performed. After the switch, pressure and flow were drastically decreased, allowing the source to work under ideal column flow conditions. Working this way, both system performance and high method throughput were maintained. (Figure 6B).

Future work will explore the combination of the Deans switch with the high-efficiency source (HES) and Agilent JetClean technology, which may further enhance routine activity and maintenance intervals.

Validation study: Overall, the validation of this method shows that, across a range of urine samples (varying in pH, density, etc.), half of the minimum required performance levels (MRPLs) can be easily reached while maintaining robustness, specificity, and selectivity. For all targets the LODs were below half of the corresponding MRPLs.

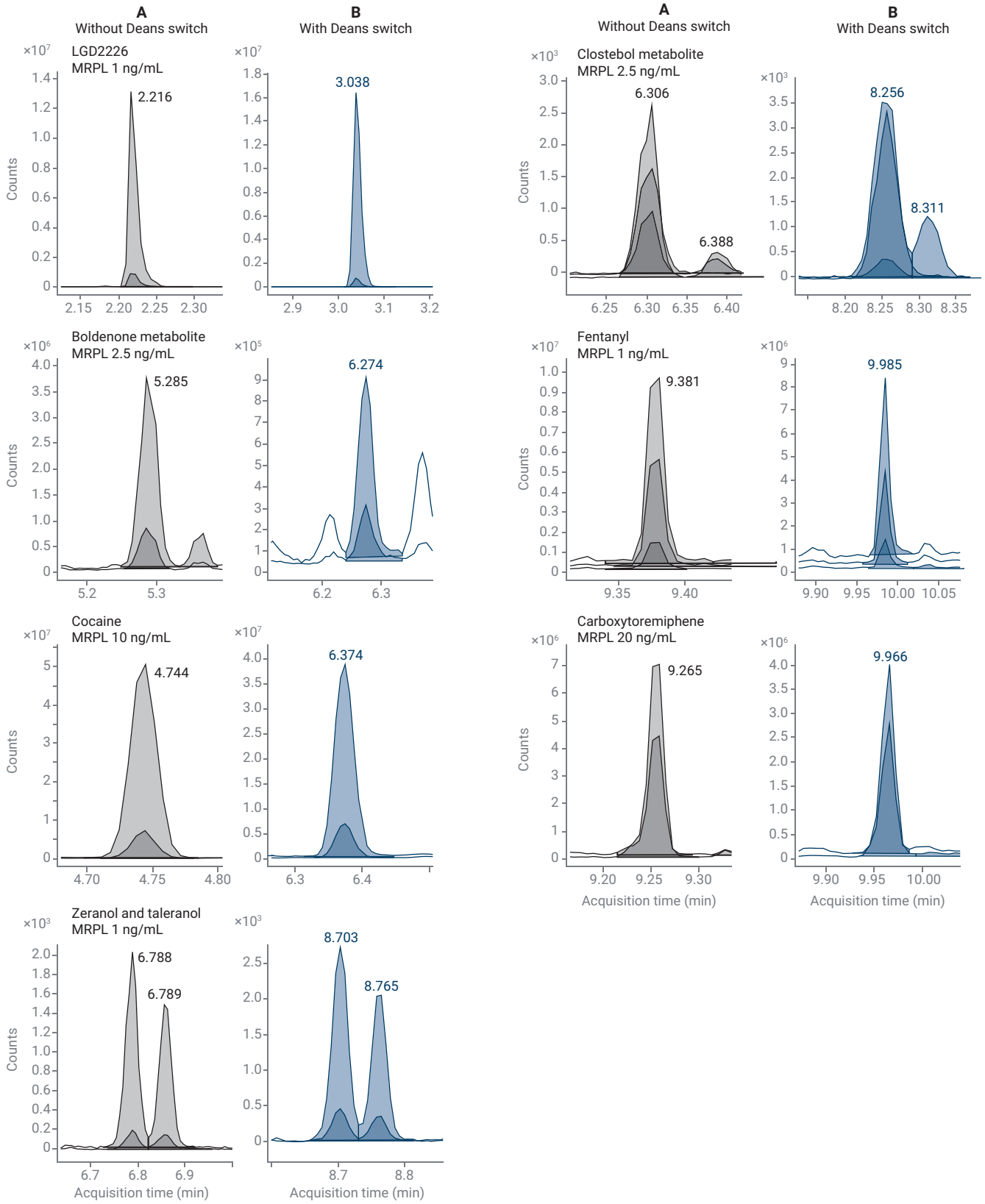


Figure 6. Targets at their respective minimum required performance levels (MRPLs), shown as (A) without a Deans switch and (B) with a Deans switch.

Conclusion

The integration of the Deans switch into high-throughput anti-doping GC/MS/MS workflows provides a robust solution to the persistent challenge of EI source fouling by derivatization agents and matrix contaminants. By selectively venting solvent and early eluting byproducts during the initial phase of the chromatographic run, the Deans switch significantly extends instrument maintenance intervals and enhances overall analytical stability. Both the Agilent 7000 Series GC/TQ with hydrogen carrier gas and the Agilent 7010 Series GC/TQ with helium carrier gas demonstrated marked improvements in operational efficiency, with up to a five-fold reduction in source cleaning frequency and consistent sensitivity maintained across extended sequences.

The WADA validation demonstrated that, for all analyzed samples, z-scores were less than 1, indicating acceptable method accuracy.

In this work, the Deans switch was introduced into two routine anti-doping workflows to address the issue of EI source contamination from derivatizing agents and column bleed. Two applications were described:

- **Confirmation and quantitation of endogenous steroids in urine using the 7000 Series GC/TQ with hydrogen carrier gas and the extractor ion source.** This method enables high-throughput analysis with extended maintenance intervals (≥ 1,500 injections) and robust chromatographic performance, even for challenging analytes present at low concentrations.
- **Routine screening of exogenous steroids and other doping agents in complex/dirty samples using the 7010 Series GC/TQ with helium carrier gas and the HES source.** This workflow maintains premium sensitivity for routine screening, while the Deans switch reduces the frequency of source cleaning by approximately 3x through venting derivatization agents during the solvent window. This capability is critical for reliable results in routine anti-doping laboratories. Notably, use of a Deans switch may result in higher flow rates to the EI source, which could affect method sensitivity. Accordingly, the method must be evaluated in terms of sensitivity at the detection limit when a Deans switch is implemented.

These benefits translate directly to increased laboratory uptime, reduced maintenance burden, and reliable quantitation of a comprehensive panel of anabolic steroids in complex urine matrices. The approach integrates seamlessly with validated sample preparation and acquisition methods, supporting the rigorous demands of routine anti-doping analysis and forensic toxicology. As demonstrated, the Deans switch is a valuable addition to modern GC/MS workflows, enabling laboratories to achieve higher throughput, improved data quality, and greater operational resilience.

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