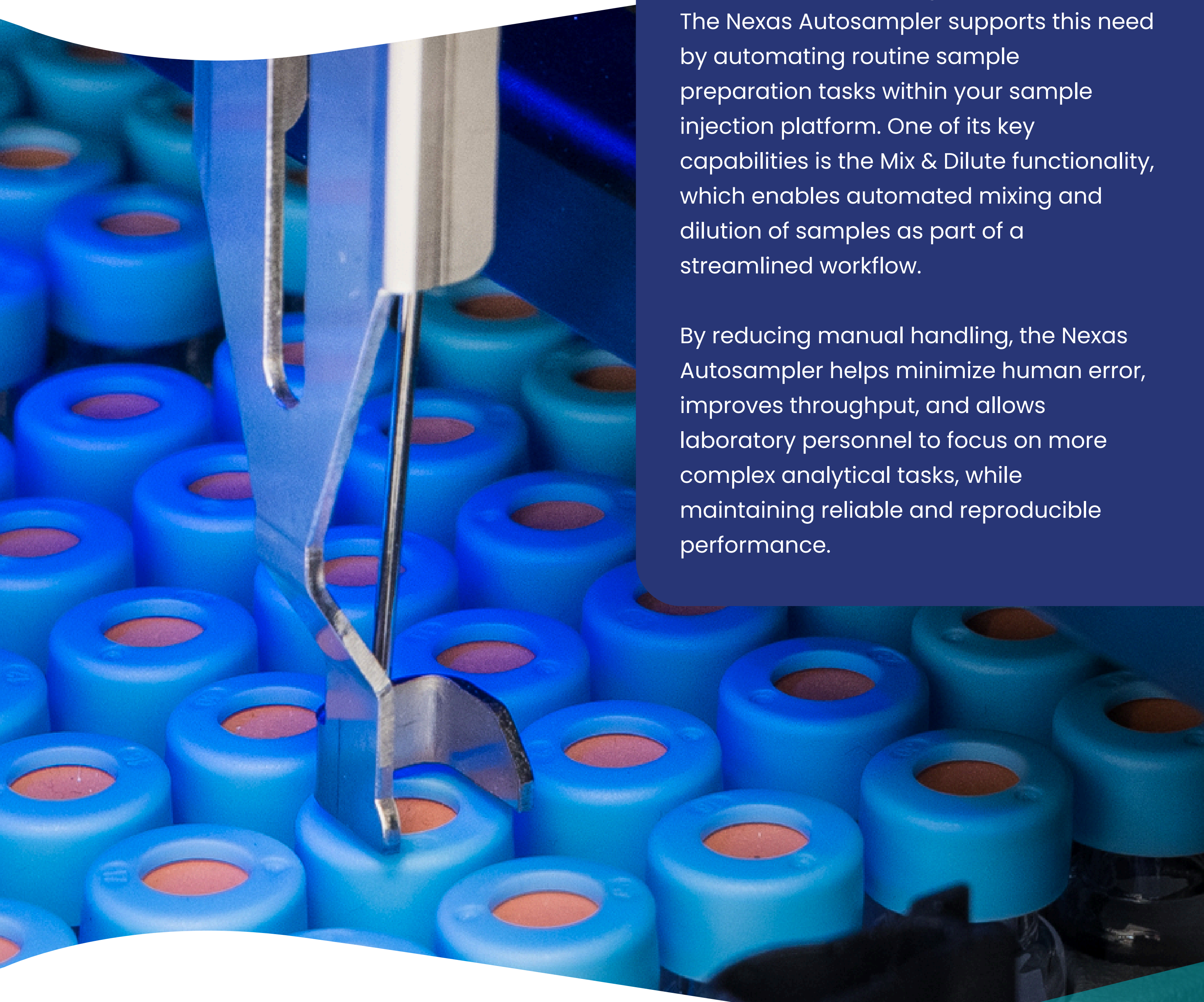
Automation Sample Preparation

Enhancing Efficiency with the Nexas Autosampler Mix & Dilute Functionality



Overview

Automation plays a key role in modern analytical laboratories by increasing efficiency and ensuring consistent results. The Nexas Autosampler supports this need by automating routine sample preparation tasks within your sample injection platform. One of its key capabilities is the Mix & Dilute functionality, which enables automated mixing and dilution of samples as part of a streamlined workflow.

By reducing manual handling, the Nexas Autosampler helps minimize human error, improves throughput, and allows laboratory personnel to focus on more complex analytical tasks, while maintaining reliable and reproducible performance.

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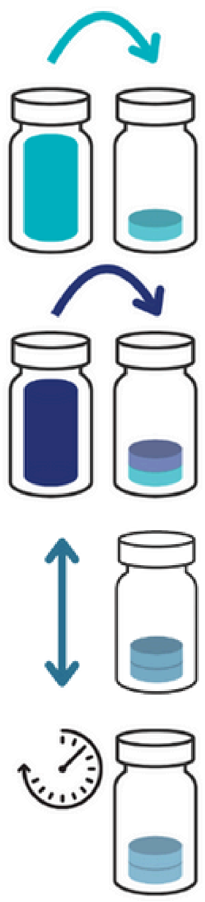
Mix & Dilute Functionality

The Mix & Dilute functionality of the Nexas Autosampler is designed to automate the preparation of diluted and mixed samples in a controlled and reproducible manner. The process consists of three fundamental steps: Add, Wait, and Mix, each contributing to accurate volume handling and proper homogenization of the sample.

The sequence of these steps can be programmed in different orders, providing flexibility to adapt the workflow to specific application requirements. In addition, the functionality can be extended for Original Equipment Manufacturer (OEM) customers, providing targeted customization options engineered to integrate seamlessly with the exact technical requirements of their solutions.

An example workflow, which is also used for generating the results presented in this technical note, is shown in Figure 1.



Add
Adding sample from source 1 to destination vial, *e.g., stock solution*

Add
Adding sample from source 2 to destination vial, *e.g., diluent*

Mix
Mixing step in the destination vial *to achieve homogenization*

Wait
Wait step or incubation step *allowing potential reaction between two substances*

Figure 1: An example for diluting a sample using the mix & dilute functionality

Add

The add step begins with aspirating an air segment to separate the wash solvent from the source solvent, thereby preventing contamination. A small volume is aspirated to flush the needle and tubing, after which the programmed solvent volume is aspirated and dispensed into the destination vial. The needle and tubing are then rinsed with wash solvent. The required reagent is subsequently aspirated and dispensed, followed by a final rinse to ensure proper system conditioning.

Mix

The mix step ensures uniformity in the sample. An air segment is aspirated to avoid contact with any wash solvent in the needle, followed by the aspiration and dispensing of sample back into the same vial or well. Finally, the needle and tubing are rinsed with wash solvent to ensure cleanliness before proceeding.

Wait

The Nexas can introduce a programmable waiting period within this functionality. This step allows the prepared solution to equilibrate, which may be desired when working with solutions of varying viscosities, and helps ensure reproducible conditions prior to subsequent operations.

In addition, the Wait functionality can support applications involving time-dependent sample preparation steps, such as controlled incubation or reaction delays, when required by the method.

Performance Evaluation: Dilution Accuracy and Precision

In the following section, we demonstrate the performance of the Nexas system in terms of dilution accuracy and precision, showing that it consistently delivers reliable results across various sample concentrations. The injection method used is summarized in Table 1.

Parameter	Value
Sample	Caffeine
Column	No column; restriction tubing (10 m 0.25 mm ID blue PEEK)
Mobile Phase	20 % Methanol / 80 % LC-MS water
Flow Rate	0.500 mL/min
Stop Time	3.00 min
Injection Volume	5 µl
Detection	273 nm

Table 1: Injection method for caffeine sample.

Precision

To illustrate the system's ability to reproducibly perform dilutions, despite multiple steps that could increase the margin of error, a 1:1 dilution was programmed using a standard Mix & Dilute method (as shown in Figure 1).

The Nexas Autosampler was programmed to add 30 µL of a 200 ppm caffeine stock solution in LC-MS grade water from source 1 to the destination vial, followed by 30 µL of LC-MS grade water from source 2. The sample was subsequently mixed by aspirating and dispensing 30 µL of the solution nine times within the destination vial, as defined in the method.

This procedure was performed using 12 separate vials, after which 5 µL from each vial was injected for RSD calculation. The complete sequence was repeated in triplicate to assess reproducibility.

The control condition consisted of 12 injections from a 100 ppm sample prepared in separate vials, also performed in triplicate.

For both conditions, the mean RSD and standard deviation (SD) were calculated. A two-sample t-test assuming unequal variance showed no statistically significant difference between the conditions ($p = 0.235 > \alpha = 0.05$), indicating comparable precision.

	100 ppm caffeine standard	**Mix & Dilute**
	0.19%	0.17%
	0.04%	0.23%
	0.14%	0.18%
Mean RSD (%)	**0.12%**	**0.19%**
SD (%)	**0.07%**	**0.03%**

Table 2: Precision in percentage for both the 100 ppm caffeine standard and the Mix & Dilute 100 ppm measured samples (n = 12).

The precision was calculated by taking the percentage of the standard deviation over the average peak area. Table 2 shows that, in comparison to the standard 100 ppm samples, the precision of the Mix & Dilute method is similar. Figure 2 displays an overlay showing that the peaks align well in terms of area.

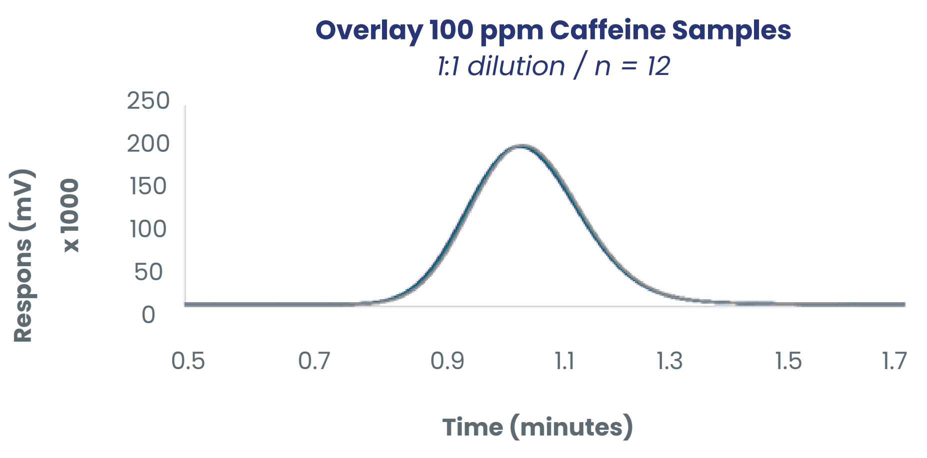


Figure 2: Overlay 1:1 diluted caffeine samples using the Nexas Mix & Dilute functionality.

Dilution accuracy

To evaluate the ability of the Nexas system to generate reliable calibration curves, a dilution series ranging from 20 ppm to 200 ppm was prepared in 20 ppm increments. The Mix & Dilute functionality was used to prepare each sample directly in the vial with a total volume of 60 µL per well, using LC–MS grade water as the diluent. The same stock solution was used as in the precision experiments

Calibration levels were prepared by combining defined volumes of stock solution and LC–MS water (e.g., 6 µL stock and 54 µL water for 20 ppm; 12 µL stock and 48 µL water for 40 ppm), demonstrating accurate dilution with a minimum volume resolution of 6 µL. A blank sample (0 ppm) was prepared by combining LC–MS water with LC–MS water.

Peak areas were plotted against concentration to construct calibration curves, which were generated in triplicate to assess reproducibility.

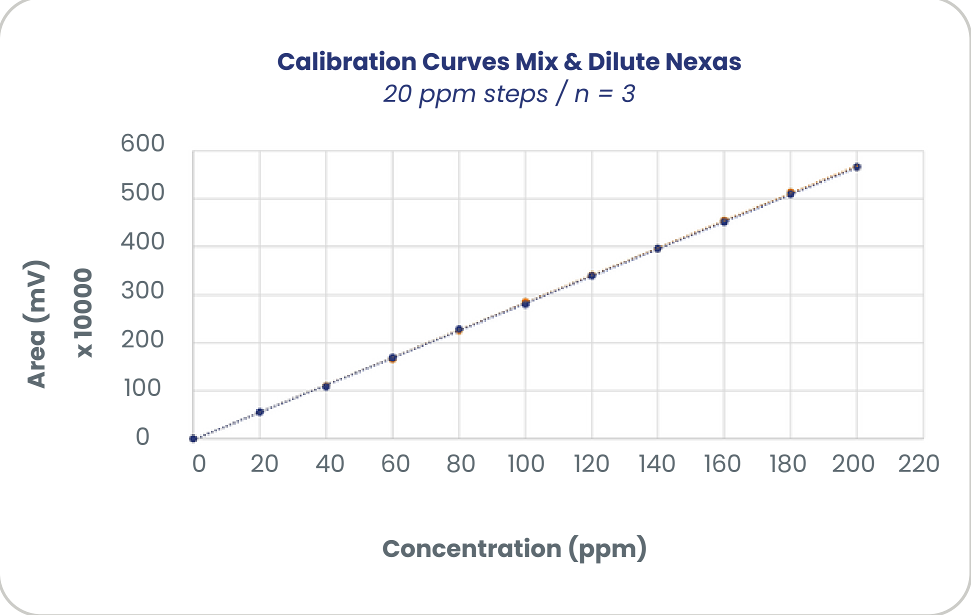


Figure 3: Calibration curves generated using automated sample dilution with the Nexas Mix & Dilute function, presented in triplicate.

The results, shown in Figure 3, highlight the Nexas system's performance, generating three calibration curves that align with one another and all exhibiting an R-squared value of >0.9999. The system consistently maintained the 6 µL volume difference between each step, demonstrating its ability to provide precise, accurate, and highly reproducible results, making it a reliable choice for a broad spectrum of analytical applications

Conclusion

The Mix & Dilute functionality of the Nexas autosampler significantly enhances efficiency and accuracy in sample preparation.
By automating dilution and mixing processes, it reduces variability and risk by limiting manual effort and human error, ensuring reproducible and precise results. The system's ability to generate calibration curves with an R-squared value of 0.9999, combined with consistently low precision values below 0.3%, demonstrates its exceptional performance in achieving both dilution accuracy and repeatability. With its flexibility and reliability, the Nexas Autosampler is a valuable tool for laboratories seeking to improve throughput while maintaining high-quality outcomes.