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Poster Presentation

Fully Automated Sample Preparation and Nanoelectrospray MS Analysis using ZipTips®, the Precision 2000™ and the NaoMate™100

Abstract

Today's increased throughput demand for Nanoelectrospray Mass Spectroscopy (MS) has driven the need for automation. In addition to automating the loading of samples into theMS, automation of the sample preparation, such as desalting, purification and concentration ofthe samples, is necessary to provide the throughput required. Here we describe a simple procedure that employs the use of the Precision 2000™ Automated Multi-channel Pipettor, inconjunction with ZipTips® pipette tips to perform the pipetting tasks required for sample preparation prior to automated nanoelectrospray mass spectrometry using a NanoMate™ 100.

The Precision 2000 ™ is an automated pipettor that uses an 8- or 12-channel multi-channel pipette arm to pipette fluids anywhere on a completely configurable multi-station deck. The pipette armmoves up and down as well as side-to-side, while the platform moves front to back to provide complete access to all locations on the work platform. The pipette arm uses a proprietary technology to reliably pick up and seal any standard tip with individual, free-floating barrels that compensate for tips out of position. The Precision 2000 ™ handles ZipTips, which are used to desalt samples. ZipTip pipette tips are 10-microliter pipette tips that contain 0.6 microliters of immobilized chromatography media (C18) in the distal ends. The beads are immobilized in a membrane scaffold that has been formed within the pipette tip housing. The tip serves as a miniature chromatography column for microscale solid phase extraction(SPE). After sample preparation, the NanoMate, an automated nanoelectrospray infusion system, and a mass spectrometer analyze the ZipTip effluent. The NanoMate 100 is a unique, fully automated nanoelectrospray system that easily mounts to a variety of mass spectrometers. Sample aliquots (1 to 10 microliters) are drawn into disposable conductive tips from a standard 96-well plate. Each sample is processed using a separate tip and nozzle, so there is no carryover between samples.

A comparison of results between the manual method and the use of the Precision 2000™ to automate sample preparation is presented.

BioTek Instruments, Inc., P.O. Box 998, Highland Park, Winooski, Vermont 05404-0998 USA

TEL: 888-451-5171 FAX: 802-655-7941 Outside the USA: 802-655-4740 E-mail: customercare@biotek.com

www.biotek.com

Introduction

Mass Spectrometry (MS) has become increasingly popular tool in biomedical research. Mass Spectrometry can be used for a number of different applications including the quantification and identification of peptides, amino acids and other small molecules. As a result, a large amount of effort has been made to improve and automate the process of mass spectrometry. The NanoMate™ 100 (Advion BioSciences, Ithaca, NY), in conjunction with the ESI chip, have been shown to provide improved sensitivity, eliminate sample cross contamination, as well as automate the loading of samples into Mass Spectrometers. However, automation of the sample preparation, such as desalting and concentration, prior to MS has historically been performed manually or required the use of large expensive pipetting devices. The Precision 2000™ Automated Pipettor (BioTek Instruments,Inc., Winooski, VT) has been developed as a low-cost high-value automated multi-channel pipettor to meet many of today's pipetting challenges. Using ZipTips® with C18 media (Millipore Corp. Bellerica, MA), the Precision 2000™ has been able to automatically desalt and concentrate samples prior MS. Here we describe the overall process, as well as demonstrate the ability of the Precision 2000™ to precisely and repeatibly pipette as compared with manually performed sample preparation.

The Precision 2000™ has a completely configurable six-station platform to hold the equired pipette tips, reagent troughs, and microplates (96- and 384-well) for fluid transfer (Figure 1). The platform is removable, allowing for multi-user friendliness, easy cleaning, and setup of the instrument. The 8- or 12-channel pipette arm moves up and down as well as side to side, while the platform moves front to back to provide complete access to all locations on the work platform with full configurability. The pipette arm utilizes a proprietary technology to reliably pick up and seal any standard tip with individual, free-floating barrels that compensate for tips out of position (Figure 2). An optional rapid dispense 8- or 12-channel manifold, which uses a precise bi-directional syringe pump to accurately and rapidly dispense fluids from a large unpressurized reservoir, is also available. The Precision 2000™ has a built in microprocessor that controls all movements. The flexible onboard software, which provides complete programming for the most complex fluid transfers, can store up to 80-programmed assays. For more complete automation robotics, interfaces can be developed using ActiveX® software commands. The Precision 2000's small size, with a 15 x 21-inch footprint and a height of 16 inches, allows it to be used almost anywhere including most biological safety cabinets or chemical fume hoods. Also available is an optional aerosol cover for use on the laboratory bench.

The NanoMate™ 100 is a unique, simple-to-use, automated nanoelectrospray platform using the patented ESI Chip™ to increase the sensitivity, throughput, and quality of chemical and biochemical analysis using mass spectrometry. The NanoMate™ 100 nanoelectrospray system easily mounts to triple quadrupole, ESI-TOF, QTOF, FT/MS, and ion trap mass spectrometers (Figure 3). Sample aliquots (1 to 25 µl) are drawn into disposable conductive tips from a standard 96-well plate. Each sample is processed using a separate tip and nozzle, so there is no carryover between samples. A sample-filled tip aligns with a chip nozzle, creating a tight seal. Nanoelectrospray initiates when head pressure and voltage are applied through the sample tip. The system infuses then moves to the next sample automatically. The 100-nozzle array of the disposable ESI Chip™ provides 96 nozzles for samples plus 4 additional nozzles for system testing and tuning (Figure 4).

ZipTips® are 10-µl pipette tips that contain 0.6 µl immobilized chromatography media (C18) in the distal ends. The beads are immobilized in a membrane scaffold that has been formed within the pipette tip housing. The tip serves as a miniature chromatography column for microscale solid phase extraction. Although several types of chromatography beads have been immobilized, the use of C18 for desalting and concentrating samples prior to MS analysis is the most common.

The ZipTip$_{(\mu\text{-C18})}$ used for these experiments (catalog # ZTC1 8PO 96) has been designed to work with an adapter press-fit into the ZipTip to accommodate automated pipettors such as the Precision 2000™. The configuration used incorporated an adapter for use with the Precision 2000™ that was removed prior to dispensing into the NanoMate.

Materials and Methods

For a calibration curve, dilutions of alprazolam standards (1.5, 2.0, 3.0, 10, 25, 100, 250, and 500 ng/ml) were prepared in plasma (dog) from a 100 µg/ml stock solution. For repeatability experiments, quality control samples of 10, 250 and 400 ng/ml of alprazolam in dog plasma were prepared. To each standard or quality control sample a fixed amount (6 ng) of alprazolam-5d was added as an internal standard. Samples were then centrifuged to remove particulates and 100 µl of the supernatant dried to completion. Samples were then reconstituted in 20 µl of 0.1% trifluoroacetic acid (TFA) without vortexing. A microplate containing the individual reconstituted samples was then placed on the Precision 2000™ for automated processing.

The deck of a Precision 2000™ Automated Multi-channel pipettor was configured to desalt the previously described reconstituted samples. Necessary reagents were loaded into troughs at position B of the Precision 2000 deck, ZipTips with Packard compatible adapters were located in position A, while conical PCR microplates were located at position E (raw sample) and position C (target for desalted samples) of a Precision 2000™ Automated pipettor (Figure 5). Using the Precision 2000™ to pick up tips, as well as pipette reagents and samples, the ZipTips were first washed with methanol by aspirating and dispensing 20 µl of 100% methanol from a reagent trough in position B seven times. The ZipTips are then equilibrated with 0.1% trifluoroacetic acid (TFA) by aspirating and dispensing 20 µl of 0.1% TFA several times from another reagent trough containing the solution also in position B. The sample, located in PCR microplate located in position E, was then loaded onto the ZipTip by repeated aspiration and dispensing into the well for a total of 10 cycles. The ZipTips with bound samples were then washed by aspirating 20 µl of 0.5% formic acid from a reagent reservoir at position B and dispensing the wash material into a waste reservoir two times. Samples were then eluted from the ZipTips by aspirating elution buffer (75% methanol, 0.1% formic acid) from its reagent trough and dispensing the eluate into wells of a PCR microplate located at position C of the Precision 2000 deck.

Sample analysis was performed with a NanoMate 100 and ESI Chip automated nanoelectrospray system attached to a API 3000 mass spectrometer with a 2-cm desolvation chamber. The NanoMate sequentially aspirates samples from a 96-well microplate using disposable conductive tips. The NanoMate delivers the sample to the inlet side of an ESI Chip ™, which consists of a 10 x 10 array of nanoelectrospray nozzles. Spray voltage (1.3 kV) and head pressure (0.3 psi) are applied to the liquid sample, creating a nanoelectrospray plume from the chip nozzle.

Sample desalting and concentration

Wash Millipore ZipTip with 100% methanol
Equilibrate ZipTip with 0.1% TFA
Load sample
Wash ZipTip with 0.5% formic acid
Elute with 75% methanol/0.1% formic acid

Mass Spectrometry

SRM Transitions Monitored

Alprazolam:	m/z = 309.2 → m/z 281.1
Alprazolam-d5:	m/z = 314.2 → m/z 286.1

Nano-ESI/MS Analysis

NanoMate 100 and ESI chip
API 3000 Mass Spectrometer
Spray Voltage: 1.3 kV
Pressure: 0.3 psi
Infusion setting: 5 sec
Equilibration time: 3 sec
Total Acquisition Time: 18 sec
Acquisition software: Analyst 1.2

Results

Using the Precision 2000™ with ZipTips to automatically perform the sample preparation results in equivalent results when compared to the results when the task was performed manually (Table 1). The analyte to internal standard ratio for 8 different 400 ng/ml samples pipetted with a Precision 2000™ had a mean of 1.814 and a CV of 1.693%, while those pipetted manually had a mean of 1.835 and a CV of 2.348% respectively. When quantitative measurements are required, the Precision 2000™ was capable of yielding quantitative results when a series of standards were processed and analyzed (Figure 6). When the ratio of analyte (Alprazolam) to internal standard (Alprazolam-d5) is plotted against Alprazolam concentration a linear relationship is observed. Concentration determinations can be made with a high degree of confidence as the correlation coefficient (r^2) for the linear regression analysis was found to be >0.999. QC control samples at three different concentrations were also processed with the Precision 2000™ and quantitated using a calibration curve using the same method (Table 2). The results with these samples were also very precise, repeatable and accurate, with CVs less than 5%.

Discussion

These data demonstrate that the Precision 2000™ Automated Multi-channel Pipettor is capable of automating the sample preparation tasks required for use of the NanoMate 100 nanoelectrospray device in conjunction with Mass spectrometry. The Precision 2000 offers a low cost alternative to automated pipetting. While not a complete “turn-key” start-to-finish automation solution, the combination of Precision 2000 and the NanoMate 100 requires minimal user intervention. After loading the deck of the Precision 2000, the user only needs to remove the processed sample plate and load it into the NanoMate for MS analysis. This step could also be automated using a robotic arm to transfer the plate from one device to the other if throughput requirements demanded.

Summary

Automated desalting using the Precision 2000 and ZipTips is equivalent to manual method

Nano-ESI/MS analysis using the Precision 2000 with ZipTips yielded quantitative responses for standards

Automated sample preparation using the Precision 2000 was equivalent to manual procedures

Use of the Precision 2000 produced repeatable results for both standards, as well as the QC Controls

Figure 1. Precision 2000 Automated Multichannel Pipettor with Optional Aerosol Cover.

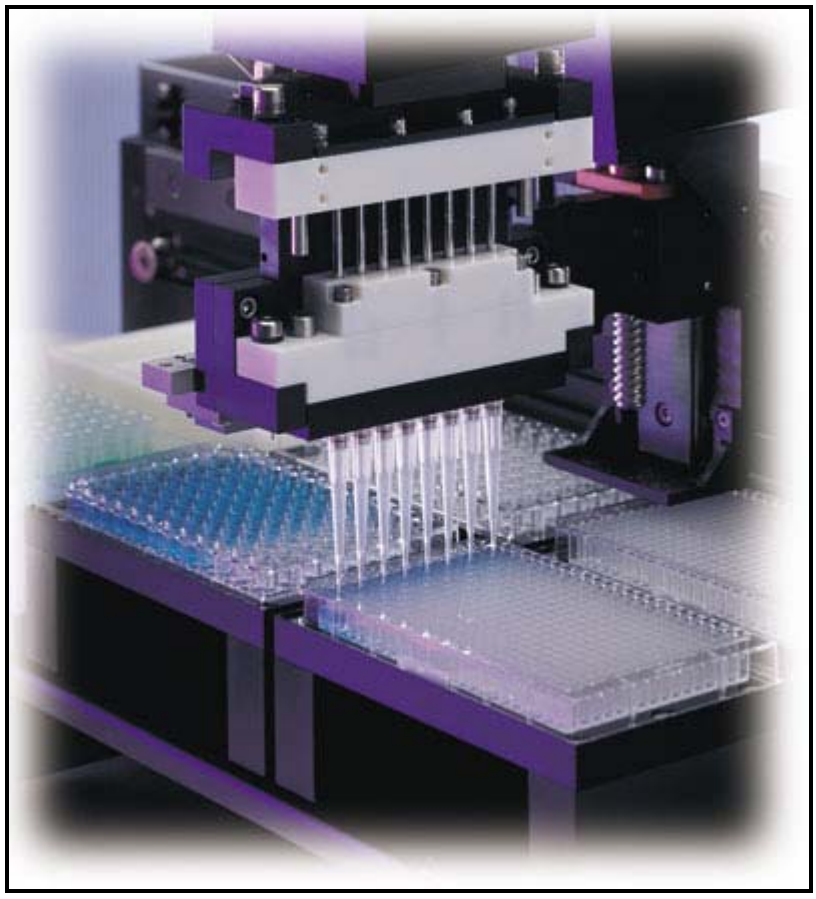

Figure 2. Pipette Arm of the Precision 2000™ Automated Pipettor with Pipette Tips. The Precision 2000™ picks up disposable tips from any location on the deck in order to aspirate and dispense fluids from either microplates or from reagent reservoirs.

Figure 3. NanoMate 100 Automates Nanoelectrospray System attached to a Mass Spectrometer.

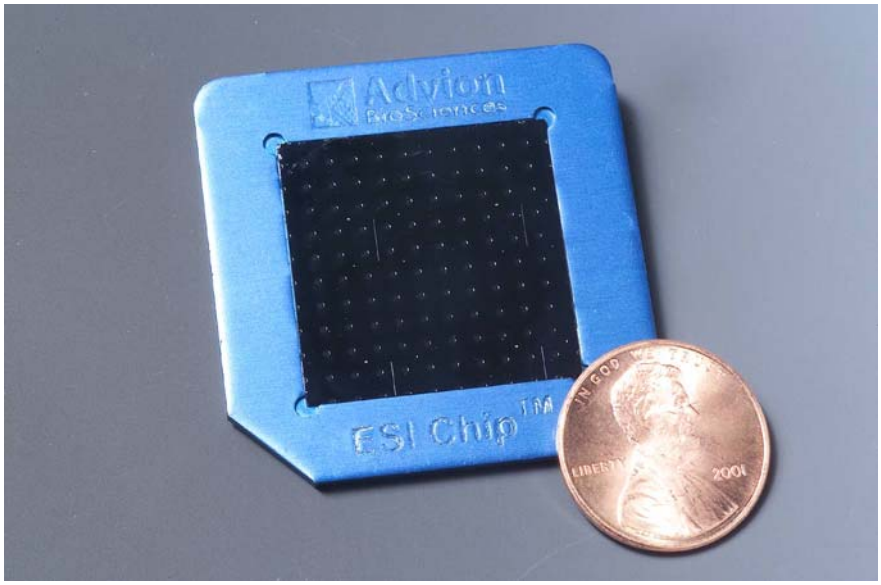

Figure 4. ESI Chip (Nozzle-side view) with a penny for size comparison.

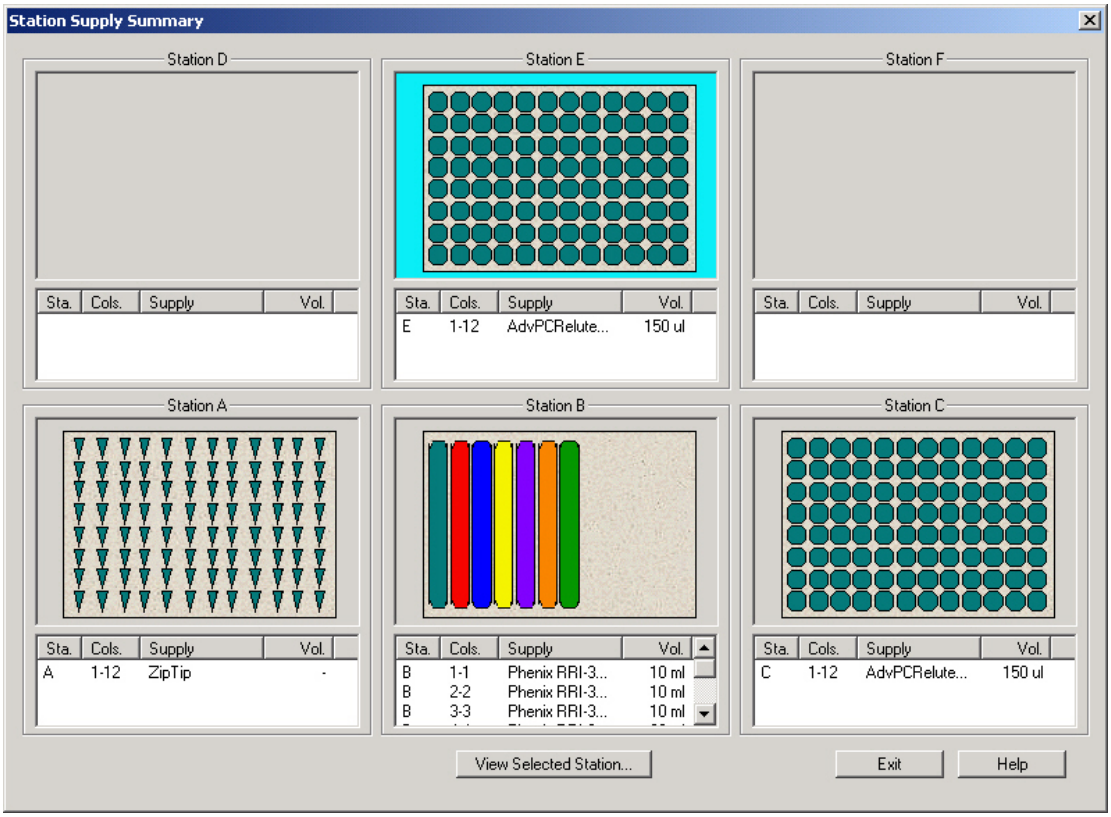

Figure 5. Deck layout of the Precision 2000 Automated Pipettor. A 96-tip box of ZipTips is mapped to position A, while necessary reagents are mapped to reagent troughs located at various locations at position B. Samples to be extracted are located in wells of a PCR plate in position E with extracted samples being eluted from the ZipTip into wells of a PCR plate located in position C.

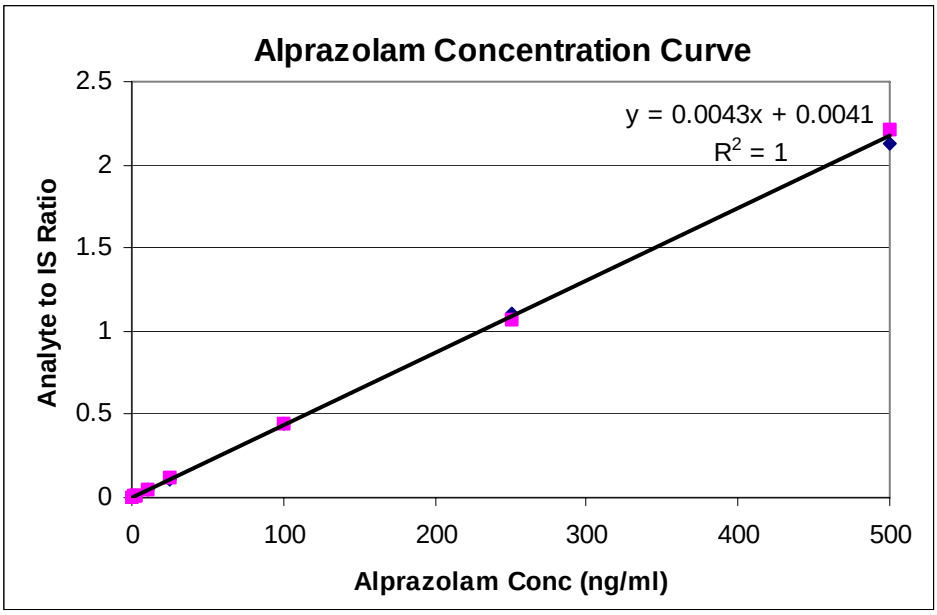

Figure 6. Alprazolam Concentration Curve. Concentrations of Alprazolam in plasma ranging from 0 to 500 ng/ml were analyzed using mass spectrometry. Using an internal standard to normalize data, the ratio of analyte standard to the internal standard was plotted against the analyte concentration and a linear regression analysis performed.

Precision 2000™ ZipTip vs. Manual ZipTip Extraction

	Analyte/IS Ratio	
#	Precision 2000	Manual
1	1.79	1.84
2	1.81	1.82
3	1.81	1.82
4	1.83	1.77
5	1.83	1.88
6	1.86	1.81
7	1.76	1.91
8	1.82	1.83
Mean	1.814	1.835
Std. Dev	0.0297	0.0431
%CV	1.639	2.348

Table 1. Comparison between manual and automated Precision 2000™ methods of ZipTip extraction. Equivalent alprazolam samples, with an internal standard alprazolam-5d were extracted manually with a hand-held multichannel pipette to move fluids or automatically with an 8-channel Precision 2000™. Extracted samples were then quantitated using mass spectrometry. Data presented represents the ratio of the analyte to the internal standard.

Alprazolam Quality Control (QC) Samples

Expected	Analyte/IS Ratio	Determined	Mean	Standard Deviation	%CV	Accuracy (%)
	0.0470	9.907				
	0.0458	9.630				
10	0.0461	9.699	9.990	0.462	4.62	99.90
	0.0508	10.784				
	0.0471	9.930				
	1.07	246.03				
	1.15	264.49				
250	1.17	269.11	258.49	9.74	3.77	103.4
	1.14	262.18				
	1.09	250.64				
	1.96	451.45				
	1.96	451.45				
400	1.99	458.38	450.99	5.00	1.11	112.7
	1.93	444.53				
	1.95	449.14				

Table 2. Accuracy and Repeatability of QC samples Processed with the Precision 2000™. Five replicates of three different quality control samples were assayed and the concentrations determined by interpolation from a standard curve. The accuracy and precision for each QC sample was then calculated.

Paul Held
BioTek® Instruments, Inc., Winooski, VT

Ellen Pace
Advion BioSciences, Inc., Ithaca, NY,

Kevin HoweTim Blankenship
Millipore Corp. Bellerica, MA

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