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Bioanalysis without Sample Cleanup or Chromatography: The Evaluation and Initial Implementation of Direct Analysis in Real Time Ionization Mass Spectrometry for the Quantification of Drugs in Biological Matrixes

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Two key bottlenecks in pharmaceutical bioanalysis are sample cleanup and chromatographic separations. Although multiple approaches have been developed in the past decade to either shorten or multiplex these steps, they remain the rate limiting steps as ADME (Absorption, Distribution, Metabolism, and Excretion) property screening is being routinely incorporated into the drug discovery process. In this work, a novel system incorporating an automated Direct Analysis in Real Time (DART) ionization source coupled with a triple-quadrupole mass spectrometer has been developed and evaluated for quantitative bioanalysis. This system has the capability of directly analyzing samples from their biological matrixes and therefore potentially eliminating the need for sample cleanup and chromatographic separations. A LEAP Technologies autosampler was customized to perform the automated sample introduction into the DART beam with high precision, which significantly improved the reproducibility of the method. Additional pumping was applied to the atmospheric pressure inlet on the mass spectrometer to compensate for the increased vacuum load because of the use of high-flow helium by the DART. This resulted in an improvement of detection sensitivity by a factor of 10 to 100 times. Matrix effects for a diversified class of compounds were evaluated directly from untreated raw plasma and were found to range from approximately 0.05 to 0.7. Precision and accuracy were also tested for multiple test compounds over a dynamic range of four orders of magnitude. The system has been used to analyze biological samples from both in vivo pharmacokinetic studies and in vitro microsomal/S9 stability studies, and the results generated were similar to those obtained with conventional LC/MS/MS methods. Overall, this new automated DART-triple quadrupole mass spectrometer system has demonstrated significant potential for high-throughput bioanalysis.

The integration of ADME (Absorption, Distribution, Metabolism, and Excretion) property screening into the early drug discovery process has become a standard paradigm for the pharmaceutical industry.[1] Most of these ADME screenings involve the quantitative measurement of compounds in biological matrixes, namely pharmaceutical bioanalysis. Because of the complicated nature of the biological matrixes, a sample cleanup step followed by chromatographic separations is essential before mass spectrometric analyses. These sample cleanup and chromatographic separation steps frequently become the bottleneck for bioanalysis. Collectively, the bioanalytical community has spent tremendous effort in the past decade and much progress has been made in the areas of automated liquid handling with 96 or 384-well plates,[2,3] high-speed separations,[4,5] parallel separations,[6,7] online extractions,[8,9] and so forth. Most of these approaches, however, involve more sophisticated instrumentation, and higher maintenance requirement and cost. It remains of considerable interest to explore new bioanalytical methodologies that can tolerate the complex biological matrixes by eliminating sample cleanup and chromatographic separation steps.

Direct Analysis in Real Time (DART) is a new ionization technology that was developed and first reported by Cody, Laramée, and Durst in 2005 for the ambient ionization of samples in either the solid, liquid, or gaseous state.[10] Since its introduction, it has found diverse applications in many areas, such as in reaction

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monitoring,[11] the direct analysis of TLC plates,[12] the detection of counterfeit antimalarial drugs,[13] investigation of various flavor and fragrance components, [14] and elemental composition determination in combination with high resolution mass spectrometry.[15] These applications involve qualitative analysis with non-biological matrixes. However, the use of DART for quantitative analysis of samples in biological matrixes has not yet been successfully demonstrated. Our own efforts to facilitate direct analysis of biological matrixes provided limited success initially although we were able to attribute the poor quantitative results to a lack of reproducibility of the sampling method.[16] Understanding that a more reliable sample positioner might improve the quantitative capability of DART ionization, we designed and implemented a consumable glass sampler that allowed us to more reliably position the sample in the optimal ionization position of the DART source.[17] Results generated in a semi-automated sampling configuration were sufficiently improved versus the original data that we undertook the effort to incorporate a common laboratory robot, the CTC HTC PAL auto sampler, to enable control of each aspect of sample positioning, from sampling time, path of sample introduction, and exact position of the sample relative to the DART source.[18,19] It was clear after implementing these improvements that the quantitative reproducibility could be improved through the use of mechanical devices for sample handling.

In this paper, we report the development, evaluation, and initial implementation of DART ionization mass spectrometry for the quantitative and direct determination of drugs in biological matrixes without sample cleanup or chromatography. Since a high-sensitivity triple quadruple mass spectrometer is generally required for pharmaceutical bioanalysis, an interface involving a novel gas ion separator (Vapur interface) has been developed which allowed for the coupling of the DART ion source with an Applied Biosystems/MDS SCIEX API-4000 triple-quadruple mass spectrometer. Without the Vapur interface, the API-4000 was unable to maintain stable vacuum within the limits of the normal operating pressure of the mass spectrometer defined by the manufacturer. Operation of the Vapur interface proved critical to maintaining the vacuum within the API-4000 while the DART was operating with helium as the ionization gas. It also improved the sensitivity of the system by sweeping the analyte laden carrier gas to the API inlet region thus reducing the potential for the gas to drift away into the surrounding atmosphere. To achieve the reproducibility and throughput required in bioanalysis, an automated sample introduction device has been developed. This Vapur enabled and automated DART/MS/MS system was evaluated for key analytical aspects for high-throughput bioanalysis including reproducibility, matrix effects, precision, and accuracy. The system was also used to analyze bioanalytical samples from pharmacokinetic studies and in vitro microsomal/S9 stability samples, and the results were compared to those generated using the conventional LC/MS/MS system.

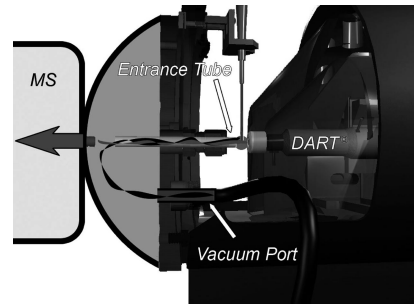

Figure 1. Schematic diagram of the Vapur interface.

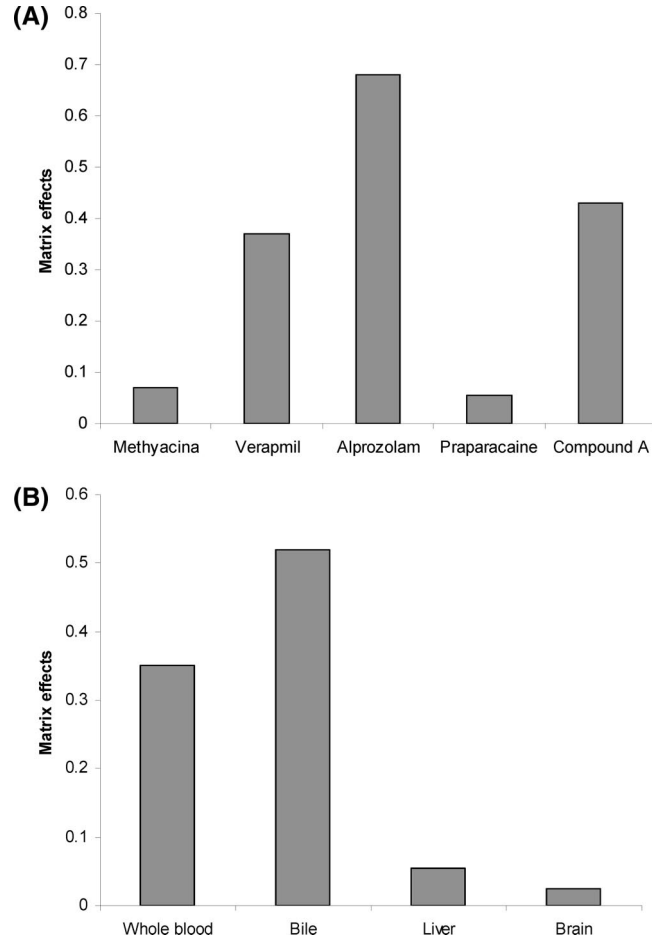

Figure 2. (A) Matrix effects of test compounds in plasma. (B) Matrix effects of verapamil in different tissue homogenates. Matrix effect is defined as the ratio of analyte peak height in matrix over that in neat solvent. A matrix effects value of 1 is no matrix effects.

EXPERIMENTAL SECTION

Materials. Blank plasma was obtained from Bioreclamation (Hicksville, NY, U.S.A.). All model compounds were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.). Proprietary

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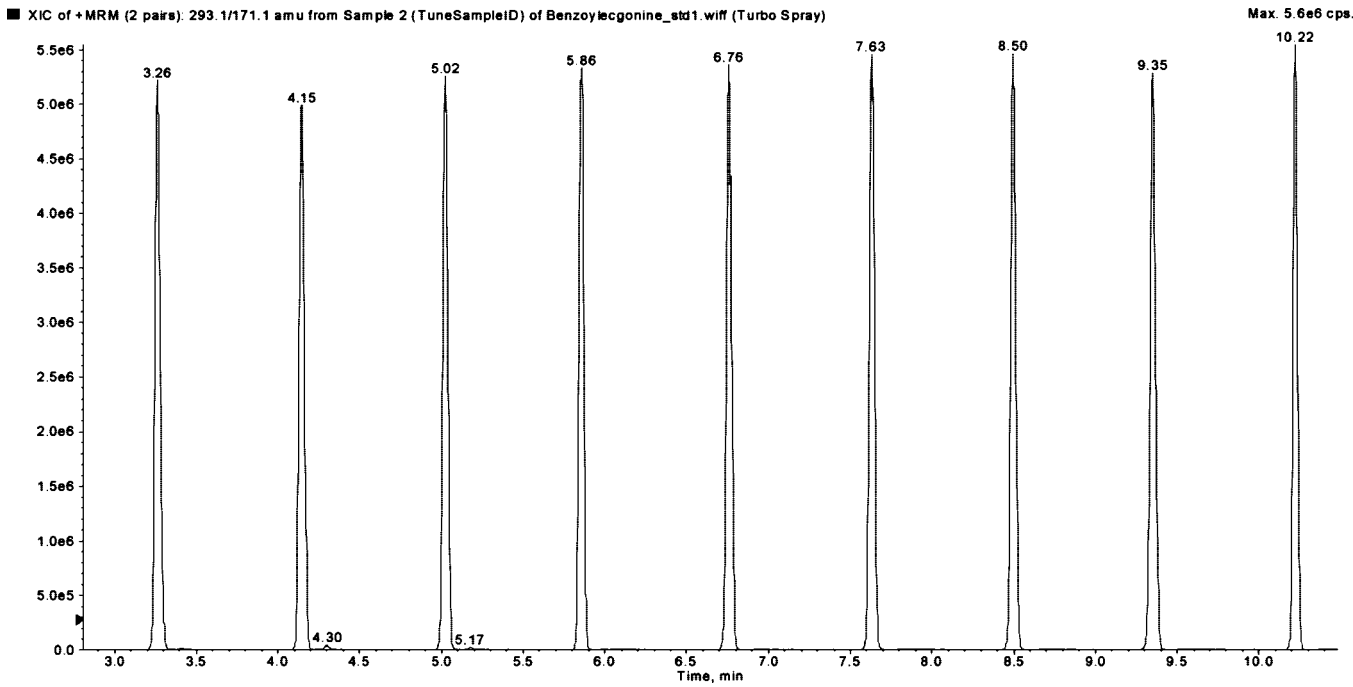

Figure 3. Reproducibility test of nine repeated injections of a rat plasma sample containing 1 uM benzoylecgonine. The %CV of the peak height is 3.1%.

Compounds A through D were synthesized in house at Millennium Pharmaceuticals Inc.

Instrumentation. The DART source completes ionization of samples by the interaction of the metastable containing carrier gas vaporizing materials from a 1 to 2 μL aliquot of sample applied to the outer surface of a glass melting point capillary (ChemGlass, CG-1841−01) which has been embedded in a formed plastic piece (DIP-it Samplers, IonSense, Inc.) and can be picked up by the action of a customized autosampler (HTCPAL, LEAP technologies), the AutoDART-96, and subsequently presented to the ionization region of the DART for desorption. The AutoDART-96 is programmed to execute a predetermined series of movements involving pickup of the sampler, dipping the closed end of the glass tube directly into the plasma, and subsequent sweeping of the glass tube through the ionization region at a rate of 500 μm per second. The temperature of the DART carrier gas was set to 425 °C to complete effective desorption of the analyte in seconds per sample. The DART source was positioned on a flat table with the AutoDART to permit reproducible desorption ionization.

Utilization of helium gas for desorption ionization with the DART presents the mass spectrometer with the requirement for greater pumping efficiency; however, physical modification of the API-4000 with larger pumping capability was not desirable. Selective removal of the helium gas from the atmospheric pressure inlet region was completed by incorporating a new vacuum chamber assembly in front of the normal API inlet and evacuating that region with a membrane pump (Vacuubrand, Diaphragm Vacuum Pump MZ 2). A schematic of the Vapur interface is shown in Figure 1. The vacuum chamber was fabricated by modifying the counter current plate with a 1/8 in. OD pump port. For the experiment, the normal counter current drying gas was eliminated since desolvation of the sample is not necessary in the DART enabled experiment. An alumina ceramic ion transfer tube (Length 9 in., OD 1/4 in., ID 4.75 mm) was used to close the gap between the DART source and the API inlet of the mass spectrometer, leaving approximately a 2 mm gap between the API-4000 skimmer and the ceramic transfer tube inside the vacuum chamber and approximately a 1 cm sampling gap between the ceramic DART cartridge and the open end of the ceramic transfer tube.

Sample Introduction Speed and Placement at the DART. The method of sample introduction at the DART was varied to determine the most effective means of sample desorption while optimizing sample to sample signal reproducibility. Two different sampling methods were programmed to run on the HTCPAL autosampler, AutoDart-96, using LEAP Shell software (LEAP Technologies, Version 3.0.1.106). The first sampling method involved sweeping the DIP-it Sampler across the entire sampling region of the DART source (1 cm) at a constant rate of 500 μm per second. The second sampling method incorporated a quick movement of the DIP-it Sampler into the center of the DART source, a short pause (5 s) with the Dip-it Sampler centered in the DART beam, and then a quick movement removing the DIP-it Sampler completely from the DART sampling region. For both sampling methods, the position of the DIP-it Sampler between the DART cartridge and the ceramic transfer tube was varied in the *y* and *z* directions.

RESULTS AND DISCUSSION

For DART to become a potential high-throughput ionization method for pharmaceutical bioanalysis without sample cleanup or chromatography, it must be able to meet some important and stringent requirements that are essential for this purpose. These requirements include reproducibility, sensitivity, linearity, and the capability of directly handling biological matrixes. The latter can be readily assessed by measuring matrix effects of test compounds in raw and untreated plasma.

Two key improvements on the instrumentation were made for this work, which were found to be critical for the use of DART

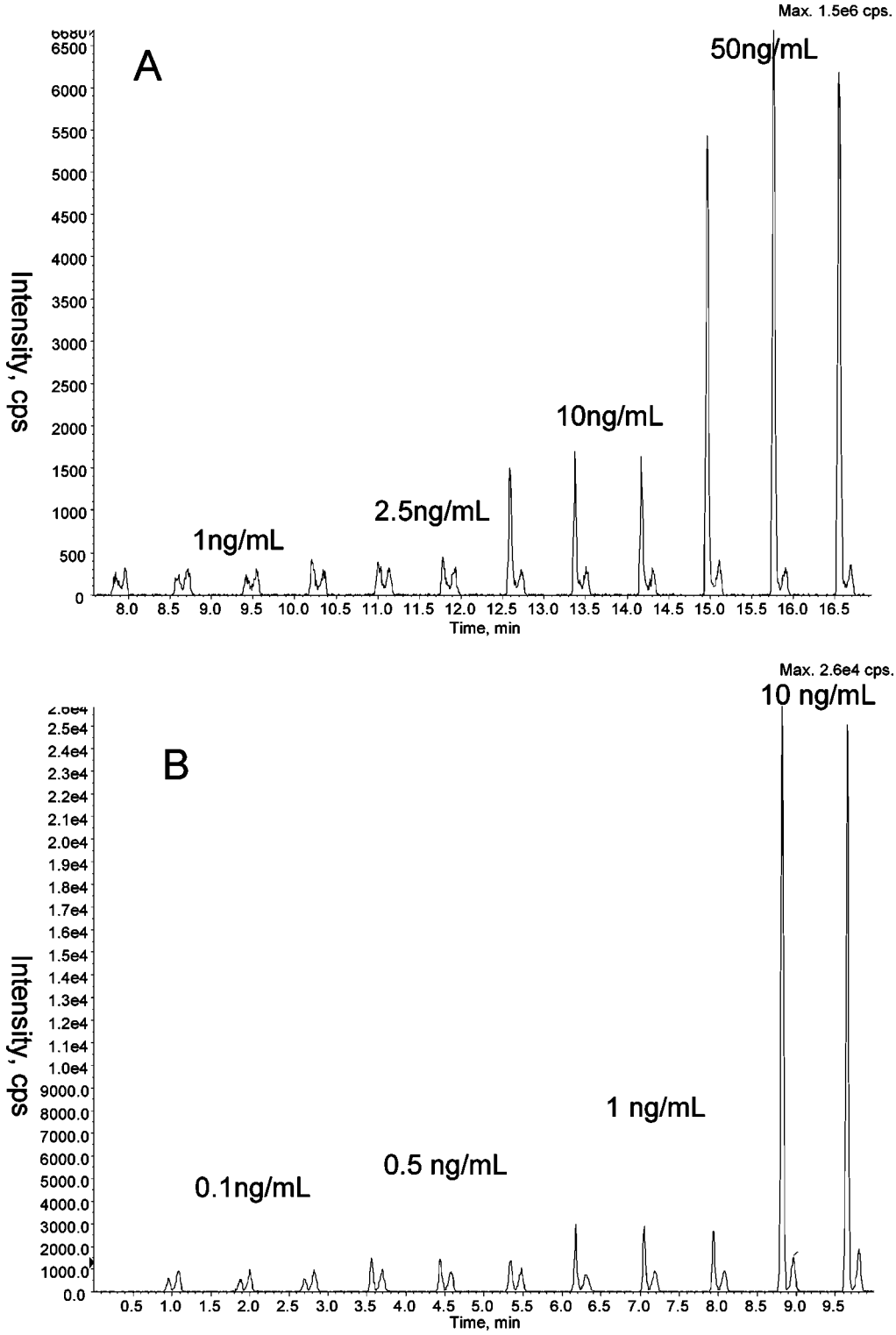

Figure 4. DART/MS/MS signals for (A) Verapamil and (B) Compound A from untreated rat plasma at different concentrations.

for quantitative measurement of drugs in biological fluids. First, implementing the Vapur interface on the API-4000 mass spectrometer provided a robust platform for DART analyses. The Vapur interface creates a low vacuum region just outside the API inlet and channels ions toward the API inlet while pulling the light-weighted helium gas away from the API inlet region. An alumina ceramic tube served as an ion channel between the DART source and the API inlet of the mass spectrometer while the inside diameter of that tube was varied. It was found that an inside diameter of 4.75 mm worked best for ion transfer. With a stabilized vacuum system it was possible to then optimize the DART/MS/MS parameters. Second, automated sample introduction was carried out and controlled using a specially programmed HTC PAL autosampler and DIP-it sampling tips providing systematic, reproducible sampling at the DART source.

Matrix Effects. To evaluate the system's capability of directly analyzing samples in plasma without cleanup, matrix effects for various compounds in rat plasma were measured. In this work,

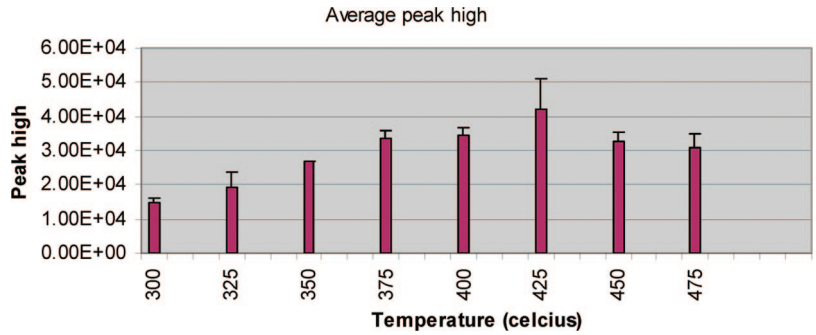

Figure 5. Average signal intensity (peak height) of DART/MS/MS as a function of DART operating temperature.

matrix effects were measured as the ratio of the analyte peak height in matrix over that in neat solution. As shown in Figure 2A, although the matrix effects were found to be compound dependent, the system was clearly able to detect all of the compounds directly from their plasma matrix. Strong matrix effects were observed with praparacaine where only 5.4% of the signal was left compared with that in neat solution. This will translate into an elevated but still practically useful quantitation limit in bioanalysis. For three other compounds tested (verapamil, alprozolam, and Compound A), the matrix effects were much less and thus had minimal impact on detection sensitivity. The matrix effects in different biological matrixes were also evaluated using verapamil as an example (Figure 2B). Different tissues showed largely different matrix effects with brain tissues representing the largest matrix effects. The implications of the strong matrix effects on assay sensitivity and potentially performance should be taken into consideration when analyzing tissue samples.

In some rare cases, when the matrix effects were too strong to detect a good signal, a sample cleanup using protein precipitation was found to be useful. One of these examples was with testosterone in rat plasma. The matrix effects of testosterone in plasma without any cleanup resulted in severe matrix effects of 0.004. After a simple protein precipitation procedure, the matrix effects were improved to 0.096.

Reproducibility. Reproducibility is one of the most important parameters for any quantitative applications. Some earlier work in our laboratory with the original DART system using a custom-made sliding sample introduction device showed limited success with suboptimal sample to sample reproducibility. Since DART is a new ionization tool which has not been demonstrated for its use in quantitative work before, an important and fundamental question is whether the reproducibility can be improved to a level that is sufficient for bioanalysis by simple mechanical modifications. Efforts were made to incorporate a LEAP Technologies autosampler to introduce the samples reproducibly into the same position in the DART beam. The glass sampling rods, that is, the DIP-it Samplers, used throughout all the experiments that are handled by the AutoDART-96 have also been made to tighter specifications by reducing variability in sampler length and diameter. These improvements resulted in significantly enhanced reproducibility. One example of the reproducibility test is shown in Figure 3. In this test, a total of nine injections of benzoylecgonine in unextracted rat plasma were made onto the system. The coefficient of variance of the peak height is about 3.1%, which is sufficient for quantitative bioanalytical work.

Sensitivity. The use of the Vapur interface has also allowed for more efficient ion transfer from the DART source to the mass spectrometer resulting in enhanced sensitivity. Figures 4A and 4B show the DART signal of verapamil and a Millennium proprietary compound (Compound A) in unextracted rat plasma at different concentrations. For verapamil and compound A, the standard of 0.1 ng/mL and 1 ng/mL can be detected with good signal-to-noise ratio, respectively. This level of sensitivity is generally sufficient for most of the bioanalytical applications. It is noted that a second and minor peak is observed for each sample. This was because when the glass sample tip passed through the center of DART beam, part of the ion signal was blocked resulting in a drop of signal intensity. Since quantitation was based on the peak height of the first and major peak, it had no impact to the result of the analysis.

Various sampling speeds using the AutoDART-96 were tested. In the range of 350 to 700 μm per second, there is no clear difference in signal intensity. Out of this range, the signal dropped significantly. Signal can be optimized with various DART temperatures as shown in Figure 5. At lower temperature, the sample will not be ionized efficiently, whereas at too high temperatures the analyte may be decomposed before it reaches the mass spectrometer.

It is worth noticing that minimal loss in sensitivity was observed when the plasma samples containing 1 uM of verapamil were diluted with water at equal volume (see Figure 6A). This was likely due to the reduced matrix effects, which compensated for the dilution. This feature is particularly useful when the sample volume is low. With further dilution (1 volume of plasma with 2 volumes of water), however, the analyte signal started to decrease (Figure 6B). The addition of 0.1% formic acid in the water during dilution slightly increased signal intensity.

Linearity. Multiple commercially available compounds with diversified structures along with a Millennium proprietary compound were evaluated for assay linearity by measuring the accuracy and precision of the back-calculated standards and quality control (QC) samples. Table 1 shows the precision and accuracy data for benzoylecgonine and a proprietary compound (Compound A) in rat plasma analyzed directly by this system without sample cleanup or chromatographic separations. For these two compounds, the %Bias for the back-calculated standards and QC samples were within ±15%, and the precision for the back-calculated standards and QC samples were within 15%. Interestingly, as shown in the table, although a stable isotope labeled compound was used as the internal standard

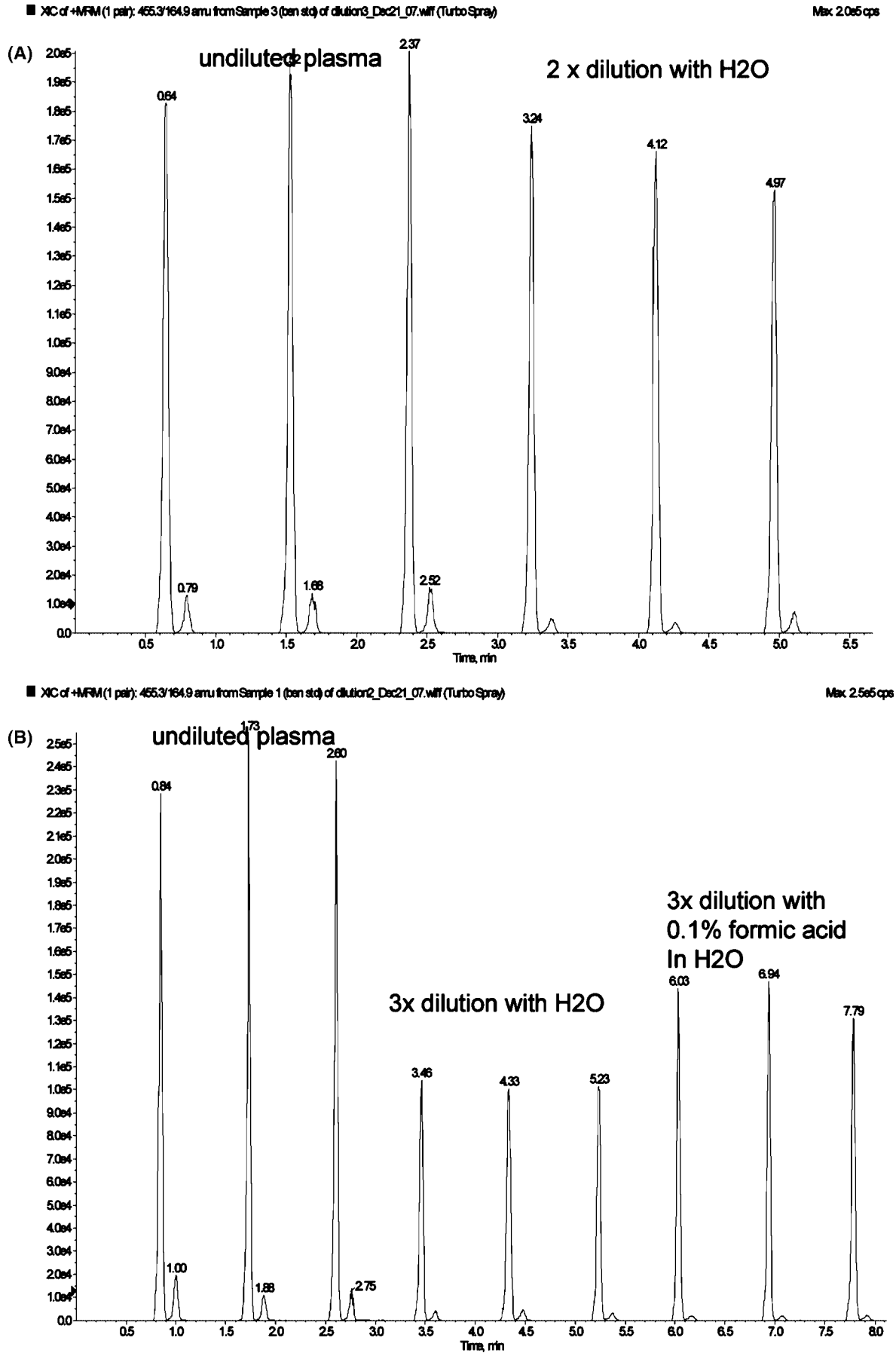

Figure 6. Comparison of DART/MS/MS signal intensity for verapamil in rat plasma. (A) The plasma sample was diluted equal volume with water. (B) The plasma sample was diluted with two volumes of waters and two volumes of water with 0.1% formic acid.

Table 1. Comparison of Precision and Accuracy of Back-Calculated Standards and Quality Control Samples of Test Compounds in Rat Plasma with and without the Use of an Internal Standard in Data Processing

concentration (ng/mL)	2	5	20	100	500	1000	2000	5000	10000	20000
					Benzoylecgonine (with Internal Standard)					
Replicate 1	2.08	4.22	20.7	101	508	1020	2000	4870	9490	19600
Replicate 2	1.12	4.65	20.0	101	512	1030	2080	4970	9750	22100
Replicate 3	2.10	4.92	20.2	101	499	993	2070	4970	9360	21300
Mean	1.77	4.60	20.3	101	506	1010	2050	4940	9530	21000
% Bias	12	−8.0	1.5	1.0	1.2	1.0	2.5	−1.2	−4.7	5.0
% CV	32	7.7	1.8	0.00	1.3	1.0	2.1	1.2	2.1	6.1
					Benzoylecgonine (without Internal Standard)					
Replicate 1	2.08	3.87	21.7	98.3	479	1020	2030	5080	9570	23400
Replicate 2	1.06	4.87	20.7	101	534	987	2140	5320	9450	21000
Replicate 3	2.14	4.69	21.1	96.9	505	927	2150	4630	9870	18200
Mean	1.76	4.48	21.2	98.7	506	978	2110	5010	9630	20900
% Bias	−12	−10.4	6.0	−1.3	1.2	−2.2	5.5	0.2	−3.7	4.5
% CV	34	11.9	2.4	2.10	5.4	4.8	3.2	7.0	2.2	12.4

concentration (ng/mL)	5	20	100	500	1000	2000	5000	10000	20000
				Compound A (with Internal Standard)					
Replicate 1	5.2	29.3	108	441	945	2250	6070	11300	22200
Replicate 2	5	17.4	78.7	498	921	1680	4740	11000	20200
Mean	5.1	23.35	93.4	470	933	1970	5410	11200	21200
% Bias	2	16.8	−6.6	−6.0	−6.7	−1.5	8.2	12.0	6
				Compound A (without Internal Standard)					
Replicate 1	5.2	26.2	104	486	884	1890	5030	11600	23200
Replicate 2	4.67	16.1	91.5	449	854	1980	4960	10900	20000
Mean	4.94	21.2	97.8	468	869	1940	5000	11300	21600
% Bias	−1.2	6.0	−2.2	−6.4	−13.1	−3.0	0	13.0	8.0

QC (ng/mL)	10	2500	16000
	Compound A (with Internal Standard)		
Replicate 1	12.1	2430	17500
Replicate 2	8.88	2550	17900
Replicate 3	16.6	2570	16900
Replicate 4	11.1	2470	17300
Replicate 5	12.3	2660	19000
Replicate 6	10.1	2710	11400
Mean	11.8	2565	16700
%CV	22.5	4.2	16
%Bias	18	2.8	4.4
	Compound A (without Internal Standard)		
Replicate 1	10.4	2650	16300
Replicate 2	9.33	2370	18500
Replicate 3	14	2590	15300
Replicate 4	12.5	2670	18900
Replicate 5	11.7	2470	17300
Replicate 6	12.9	2570	13000
Mean	11.8	2550	16600
%CV	17.1	4.5	13.3
%Bias	18.0	2.0	3.8

for each of these compounds, the precision and accuracy data generated by processing the data with and without using the internal standard were generally comparable. This finding has been consistent for almost all of the compounds tested so far. Therefore, internal standards were generally not used in this work to further simplify the procedures. Table 2 lists the precision and accuracy data for four additional test compounds including indomethacin, 7-ethoxycoumarin, terfenadine, and verapamil. All these data were obtained without using an internal standard. As indicated in the table, good precision and accuracy were achieved with these compounds. Figures 7 shows the DART signals for the standards and QC samples for verapamil as an example. A dynamic range of 3 to 4 orders of magnitude were generally achieved for these compounds.

Applications in Pharmaceutical Bioanalysis. To evaluate the feasibility of the DART/MS/MS system for pharmaceutical

Table 2. Precision and Accuracy of Back-Calculated Standards and Quality Control Samples of Test Compounds in Rat Plasma without the Use of an Internal Standard

concentration(ng/mL)	10	50	250	500	1000	2500	5000	10000
				Indomethacin				
Replicate 1	8.75	36.1	212	493	999	2510	5550	10800
Replicate 2	10.6	59.4	253.0	434	857	2580	5420	10800
Replicate 3	10.90	52	206	427	1050	2560	5360	12400
Mean	10.1	49.20	224	451	969	2550	5440	11300
%Bias	1	−1.6	−10.4	−9.8	−3.1	2.0	8.8	13.0
% CV	12	24.2	11.4	8.00	10.3	1.4	1.8	8.2
				7-Ethoxycoumarin				
Concentration (ng/mL)	10	50	250	500	1000	2500	5000	10000
Replicate 1	16.8	69.3	259	438	1040	2290	5200	9220
Replicate 2	9.5	44.2	235.0	506	915	2300	5950	9700
Mean	13.2	56.80	247	472	978	2300	5580	9460
% Bias	32	13.6	−1.2	−5.6	−2.2	−8.0	11.6	−5.4

Concentration(ng/mL)	2	5	20	100	500	1000	2000	5000	10000	20000
					Terfenadine					
Replicate1	2.15	4.72	17.7	92.8	457	1050	2270	5280	10400	21500
Replicate 2	2.01	4.51	19.0	89.7	498	1000	1990	5280	11000	19800
Mean	2.08	4.62	18.4	91.3	478	1030	2130	5280	10700	20700
% Bias	4	−7.6	−8	−8.7	−4.4	3.0	6.5	5.6	7	3.5
					Verapamil					
Concentration (ng/mL)	2	5	20	100	500	1000	2000	5000	10000	20000
Replicate 1	1.76	6	21.1	107	523	993	2160	4600	9330	17100
Replicate 2	2.04	7.3	21.9	111	523	1010	2190	4690	8440	16400
Mean	1.9	6.65	21.5	109	523	1000	2180	4650	8890	16800
% Bias	−5	33.0	7.5	9.0	4.6	0.0	9	−7.0	−11.1	−16

QC (ng/mL)	10	2500	16000
	Terfenadine		
Replicate 1	9.78	2680	11700
Replicate 2	9.81	2830	14000
Replicate 3	9.72	2710	15300
Replicate 4	9.19	2740	14300
Replicate 5	8.9	2540	15600
Replicate 6	9.07	2920	15300
Mean	9.41	2740	14400
%CV	4.3	4.7	10.1
%Bias	−5.9	9.6	−10
	Verapamil		
QC (ng/mL)	10	2500	16000
Replicate 1	9.71	2300	14200
Replicate 2	10.7	2270	13600
Replicate 3	7.95	2230	13900
Replicate 4	8.29	2490	13200
Replicate 5	7.72	2520	12800
Replicate 6	9.13	2300	13600
Mean	8.92	2350	13600
%CV	12.9	5.2	3.7
%Bias	−10.8	−6.0	−15.0

bioanalysis, the system was used to analyze samples from both in vivo and in vitro ADME studies, and the results were compared to those obtained by conventional LC/MS/MS methods.

An oral mouse PK study at 25 mg/kg was conducted with a Millennium proprietary compound (Compound B). PK samples at seven time points were collected with three animals per time point. Plasma samples were analyzed using the conventional LC/MS/MS method, as well as using the DART/MS/MS system. As shown in Table 3, the mean percentage difference of the concentrations measured with these two methods ranged from −4.7% to 16.4%, demonstrating a good correlation between the two methods.

The DART/MS/MS system has also been used to analyze samples from in vitro ADME studies. An in vitro intrinsic clearance study was conducted for two proprietary compounds in the matrixes of human S9, rat S9, and mouse microsome respectively. The hepatic extraction ratios calculated based on the data

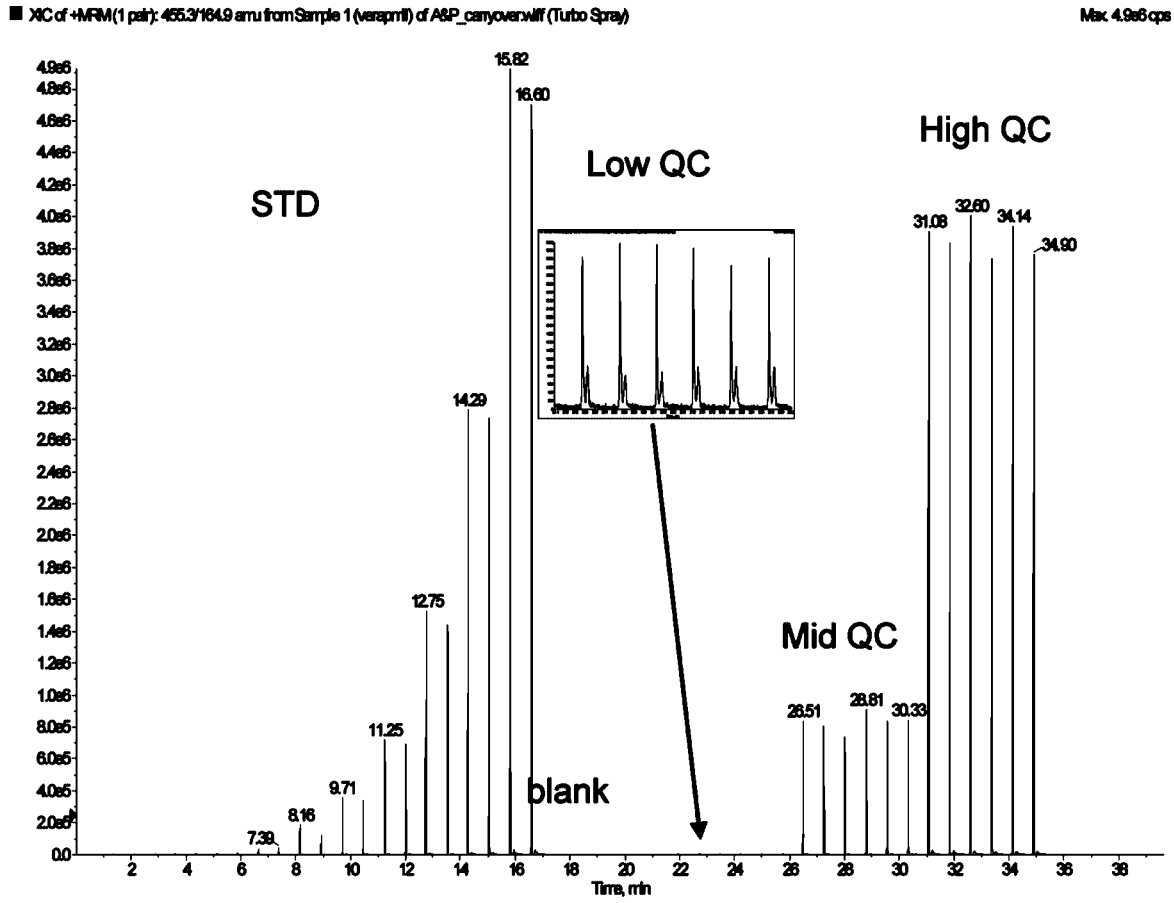

Figure 7. Representative DART/MS/MS signal for the standards and QCs of verapamil in rat plasma.

Table 3. Concentration Comparison between the DART/MS/MS and LC/MS/MS Methods for a PK Study in Mouse For Compound B

	concentration (nM)		
sample	LC/MS/MS	DART/MS/MS	%MPD
30 min (A)	3990	4170	2.2
30 min (B)	3930	4412	5.8
30 min (C)	2300	2918	11.8
1 h (A)	3500	4297	10.2
1 h (B)	4200	5846	16.4
1 h (C)	2480	3011	9.7
2 h (A)	3500	4430	11.7
2 h (B)	1890	2340	10.6
2 h (C)	2900	3073	2.9
4 h (A)	2940	2674	−4.7
4 h (B)	3480	3672	2.7
4 h (C)	4510	4766	2.8
8 h (A)	3110	2851	−4.3
8 h (B)	1900	2111	5.3
8 h (C)	3270	3130	−2.2
16 h (A)	BQL	BQL	N/A
16 h (B)	2	BQL	N/A
16 h (C)	BQL	BQL	N/A
24 h (A)	BQL	BQL	N/A
24 h (B)	BQL	BQL	N/A
24 h (C)	1.39	BQL	N/A

generated by both systems are shown in Table 4. Both methods gave similar results on the clearance of Compounds C and D. It is found that the use of internal standards helps to improve assay reproducibility for in vitro studies since the sample matrix of these in vitro samples is largely organic solvent with lower viscosity.

Table 4. Comparison between the DART/MS/MS and LC/MS/MS Methods for an in Vitro Metabolic Stability Study For Compounds C and D

	matrix	Hepatic Extraction Ratio LC/MS/MS	Hepatic Extraction Ratio DART
Compound C	Human S9	<0.19	>0.19
Compound C	Rat S9	<0.16	<0.16
Compound D	Mouse Microsomes	0.91	0.92

These less viscous in vitro samples tend to be picked by the tip (DIP-it Samplers, IonSense, Inc.) in a less uniform way from sample-to-sample compared to samples with high viscosity such as plasma samples.

Current Limitation and Future Direction. It is important to recognize some limitations of this method. From a bioanalytical perspective, the largest limitation of this method is probably the compromise on specificity because of the absence of chromatographic separations. Unlike a soft ionization method such as electrospray ionization, DART tends to break down some labile bonds in a metabolite such as a glucuronide. In our work, we have tested glucuronides of morphine and a proprietary compound. In both cases, the system was not able to detect the glucuronides at all but only the parent compounds, indicating substantial conversion of the conjugate into the parent. Therefore, this method may overestimate the parent compound if a glucuronide metabolite is present at a substantial level. Another key limitation is that the DART ion source generally only works for compounds with a molecular weight below 1000. While this feature

helps reducing ion suppression of small molecules in biological fluids, it also made the method not applicable to analyze larger molecules or biologics.

Given the unique advantage and some limitation, the DART/MS system is being further explored in the authors' laboratory in three potential areas. First, it will be used as a "real-time" bioanalytical tool for early stage exposure screening studies. Some examples of these studies are to answer whether a compound is bioavailable or to choose a formulation which provides better exposure for an efficacy study. The use of DART can provide almost a real time answer to these questions. Even if a parent compound may be overestimated because of metabolite interference, a more definitive study will be conducted to confirm the results. Second the system can be used as a high throughput tool for analyzing in vitro metabolic stability samples. The throughput of this system will allow fast metabolic screening of large number of compounds. Again any overestimate of the metabolic stability because of metabolite interference can be confirmed by more definitive studies. Finally, an extremely attractive application of the DART/MS/MS system is the use as a clinical diagnostic tool and also for therapeutic drug monitoring at the bedside. The real time analysis and the elimination of chromatography and sample extraction, particularly when combined with a portable mass spectrometer, make it potentially possible to be deployed to a routine diagnostic laboratory. This is an important application area where the DART/MS/MS can clearly distinguish itself from other conventional techniques.

Although the data from only a limited number of compounds were reported in this work, the DART technique has been used in the authors' laboratory for other proprietary compounds with more diversified structures, and the results were broadly similar with the compounds reported in this work. In a recent publication by Petucci et al.,[11] a comparison of the signal intensity between DART and electrospray in both the positive and negative ion modes were conducted for over 20 structurally diversified and pharmaceutically relevant compounds. In most cases, the signal intensity with DART was about 2 to 10-fold lower than that of electrospray, which is consistent with the results in the authors' laboratory. Although the sensitivity of DART is not as good as that of eletrospray in general, it meets the needs for the type of applications mentioned in the above paragraph, particulary when combined with a high sensitivity triple quadrupole instrument.

CONCLUSIONS

The reproducibility, sensitivity, and practicability of DART have been significantly improved with the development and modification of the instrumentation. The DART/MS/MS system was found to be adequate to meet the general requirements for non-regulated pharmaceutical bioanalysis. With some compromise in detection sensitivity and specificity, the system is capable of directly analyzing samples in biological matrixes without sample cleanup or chromatographic separation. The reproducibility, sensitivity, and assay linearity were found to be comparable or approaching those from the conventional LC/MS/MS methods. Compared to results generated with LC/MS/MS, DART/MS/MS has produced comparable results for different types of in vivo and in vitro ADME studies. The DART/MS/MS is potentially an effective tool for high-throughput and real time bioanalysis.

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