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Quantification of small molecules in plasma with direct analysis in real time tandem mass spectrometry, without sample preparation and liquid chromatographic separation

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Recently, a new ion source, Direct Analysis in Real Time (DART), has been introduced which allows direct biological sample introduction into a mass spectrometry (MS) system. The elimination of conventionally required sample preparation and separation by high-performance liquid chromatography (HPLC) prior to MS analysis represents a remarkable opportunity to reduce assay turn-around time, environmental impact and capital/manpower investment. This new technology initially was used in various qualitative applications to directly detect chemicals on solid surfaces, in liquids and gases. In this study, a DART source operating under ambient pressure with ground potential was installed onto a Sciex 4000 tandem mass spectrometer and employed in the sample analysis of plasma based on direct introduction into the DART-MS/MS system. Reasonable precision and accuracy (%CV and %Error, both <10%) were achieved of a significant number of compounds tested in biological fluids. In addition, the limit of detection for 80% of the tested compounds reached 5 ng/mL or lower which is sufficient for pharmaceutical drug discovery support. Finally, experimental conditions that significantly impacted assay performance were investigated with respect to optimization and limitation. Because of its simplicity, fast data acquisition (3–5 s) and low cost, DART has the potential to significantly impact quantitative pharmaceutical analysis in biological matrices. Copyright © 2008 John Wiley & Sons, Ltd.

While conventional liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) remains the standard bioanalytical tool, alternative techniques are constantly in development to simplify and improve sample handling and assay throughput. The first such technique, the FlashQuant workstation, was introduced in 2007 and combined a matrix-assisted laser desorption/ionization (MALDI) ion source with a triple quadrupole mass spectrometer for fast small molecule analysis.[1–3] Although LC separation is eradicated, this system requires extensive sample clean up. In addition, the sensitivity is moderate. Finally, only a small number of compounds have been tested with this system. The transfer of protons from the matrix reagent (small molecules) of MALDI to large biomolecules like proteins is efficient, but it may not occur when applied to small molecules. It has been used for some early drug discovery studies.[4–6] The second technique, Laser Diode Thermal Desorption (LDTD), was recently developed.[7] This innovative design induces rapid laser thermal desorption of the sample followed by atmospheric pressure chemical ionization (APCI). This ionization system (LDTD-APCI), coupled with a triple quadrupole mass spectrometer, has been utilized in the determination of sulfonamide residues in milk,[8] trace analysis in explosives,[9] and reserpine in human plasma.[10] Nonetheless, the requirement of this technique for sample clean up and the limitations of APCI have yet to be further evaluated. In addition, the reproducibility of LDTD-APCI-MS/MS results is unknown. Another novel ambient desorption ionization technique is Desorption ElectroSpray Ionization (DESI).[11] DESI is carried out by directing a pneumatically assisted electrosprayed jet (typically an acidic mixture in methanol/water) onto the sample surface to be analyzed under atmospheric conditions. The charged droplets pick up the sample through interaction with the surface and then form highly charged ions that can be sampled into a mass spectrometer. DESI combined with a mass spectrometer has been applied in forensics, chemistry and biology.[12] DESI-MS has also been used to qualitatively analyze pharmaceuticals,[13] drug formulations,[14] biological tissues,[15] and drug metabolites in neat solutions and urine.[16] However, no report on the quantitative analysis of pharmaceutical compounds in biological fluids with DESI-MS has been published to the best of our knowledge. Likewise, broad comparisons of sensitivity of DESI with ESI, APCI and MALDI for different compounds in biological fluids have not been reported.

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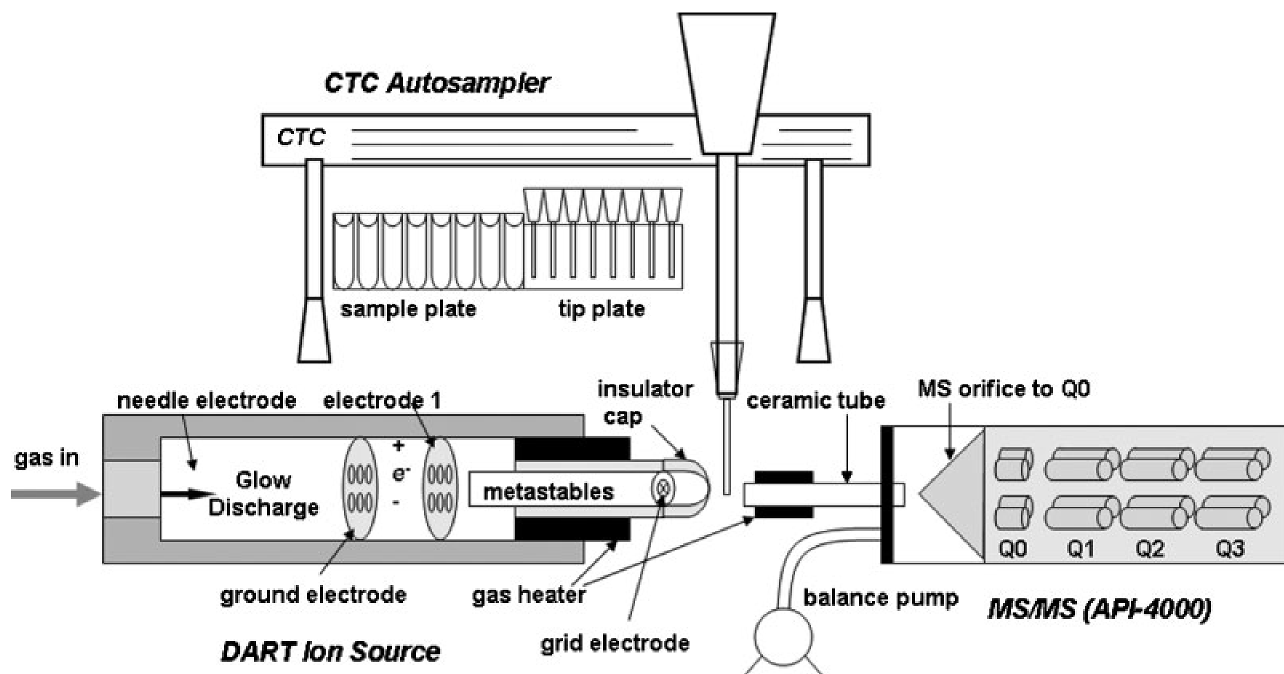

Figure 1. Diagram of the DART-MS/MS system with an autosampler.

Direct Analysis in Real Time (DART) was reported in 2005.[17,18] The DART source operates by exposing the sample to a dry gas stream (typically helium or nitrogen) (see Fig. 1). The gas stream contains long-lived electronically or vibronically excited neutral atoms or molecules (or so called 'metastables') formed in the DART source by flowing through a glow discharge in a chamber. Voltages applied to electrostatic lenses remove charged particles from the gas stream. A grid at the exit of the DART source acts as a source of electrons and reduces positive-ion/negative-ion recombination. The excited-state species can interact directly with the sample to desorb and ionize the analytes. DART mass spectra are relatively simple, dominated by protonated molecules $[M+H]^+$ in positive-ion mode, or deprotonated molecules $[M–H]^-$ in negative-ion mode. The addition of ammonia or other 'dopants' to the DART gas stream can be used to form singly charged adducts such as $[M+NH_4]^+$ or $[M+Cl]^-$ for compounds that would not readily form molecular ions or protonated molecules.[19] DART has been interfaced to mass spectrometers (time-of-flight (TOF), single quadrupole, triple quadrupole, etc.) for the analysis of counterfeit antimalarials,[20,21] formulated products,[22] chemical reactions and products,[23,24] metabolic stability,[25] fatty acid methyl esters,[26] flavors,[27] and others.[28] However, the majority of these applications are qualitative analyses, and the sample matrices are relatively simple. These technologies (FlashQuant, LDTD-APCI, DESI and DART) are still in the early stages of development and application publications are rare. However, developmental progress in these technologies will significantly impact bioanalytical chemistry.

In this report, we present the development of a DART source interfaced to a triple quadrupole mass spectrometer for quantitative analysis of small molecules in biological samples without plasma sample preparation and high-performance liquid chromatographic (HPLC) separation. Extensive evaluation and optimization were conducted on reproducibility, sensitivity, matrix effect, sample handling, device improvement, experimental conditions, etc. A comparison between LC/MS/MS and DART-MS/MS has been performed. It was demonstrated that DART-MS/MS could be utilized as a quantification tool for bioanalysis to support pharmacokinetics, toxicokinetics, pharmacodynamics, metabolism, and potentially other studies in drug discovery and development.

EXPERIMENTAL

DART-MS/MS

The details of the geometry of the DART ion source have been described previously by Cody and co-workers.[17] Figure 1 shows the configuration of the DART-MS/MS system used in our lab for this study. The DART ion source, modified CTC autosampler, balance pump and DART control computer system were purchased from IonSense, Inc. (Saugus, MA, USA). The API-4000 mass spectrometer with Analyst 1.4 software was purchased from Applied Biosystems, Inc. (Foster City, CA, USA). Helium (99.9%) was purchased from Praxai, Inc. (Danbury, CT, USA) and used for DART. The DART orifice, the ceramic tube (4 mm i.d., 10–12″), and the mass spectrometer orifice were aligned so that the stream of helium exiting the source was introduced into the orifice of the mass spectrometer to obtain the best sensitivity. The distance between the orifice of the DART source and the ceramic tube is approximately 1 cm and between the ceramic tube and the orifice of the mass spectrometer is 2 mm. The temperature of the gas heater on the ceramic tube was set at 200°C. The sample probe that holds the sample liquid scanned across the outlet of the DART source at 500 μm/s. The top of the sample probe was positioned 1/3 of the way from the bottom edge of the outlet

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orifice. The DART source and the autosampler were controlled by Leap Shell 3 and Jeol DART programs provided by Ionsense, Inc. The mass spectrometer was controlled by Analyst 1.4 software provided by Applied Biosystems, Inc. The autosampler first picked up a sample probe, dipped it into a sample solution, moved across the DART source and then disposed the used probe in a container automatically. Both positive and negative ionization and multiple reaction monitoring (MRM) were applied with the mass spectrometer. The balance pump was used to balance the pressure in the mass spectrometer to avoid too much helium flow into the orifice of the mass spectrometer. A high gas flow from the DART source into the mass spectrometer will break the pressure balance in the mass spectrometer and automatically shut it down. The ceramic tube needed periodic cleaning to maintain good sensitivity. A 96-well plate with 1 mL well volumes was used as a sample plate. The sample probes were made with 1.2 mm i.d. glass capillary or 150 μm polyimide-coated silica by IonSense Inc. and were placed on a 96-hole plate.

Chemical and solutions

All commercially available compounds and solvents, of reagent grade or higher purity, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Procedure

The optimization of the mass spectrometer for analytes using a 0.1–1 μg/mL working solution was performed with an ESI-MS/MS system. This optimization was performed using either positive or negative MRM scans. The optimized conditions of the mass spectrometer were transferred to the DART-MS/MS system to build an acquisition method. Aliquots of 0.1–0.5 mL test solutions, plasma, or other biological samples (standard, quality control (QC) or in-life study samples) were placed into the 96-well plate. For some compounds, the addition of internal standard solution (in 100% acetonitrile) followed by vortexing and centrifugation was needed. The sample plate and sample probe plate were placed onto the autosampler. Data was acquired and processed by Analyst 1.4. The pharmacokinetic analysis was done by WinNonlin 5.2 (Pharsight Corp., Cary, NC, USA).

RESULTS AND DISCUSSION

Optimization of experimental conditions

To evaluate and optimize the performance, we investigated a number of assay properties and operating parameters including compound property, sample matrix, amount of sample introduced, introduction method, surface property of sample probe, DART parameters (gas flow, temperature, voltage, etc.) and DART scan speed. The focus of the investigation was primarily on sensitivity. It was discovered that there was a strong correlation between ESI and DART signal responses for many compounds. Clean sample matrices with high volatility were associated with good sensitivity. For some compounds, water or acetonitrile was added into the plasma sample to achieve better sensitivity (see Table 1). Adding NH_4OH to compounds that only have hydroxyl or carboxyl functional groups appeared to promote the formation of MNH_4^+ adduct ions which in turn offered higher sensitivity than MH^+. Gas heater temperature and helium gas flow were two major operating parameters evaluated for optimal performance. The experimental results are shown in Figs. 2 and 3. Higher temperature and gas flow would promote desolvation and the entry of analyte molecules into the gas phase, consequently increasing the sensitivity. However, too high a temperature would cause the plasma sample to dry and char immediately and as a

Table 1. Summary of limits of detection (LODs) for commercial compounds tested (S/N = 3)

Test Compound	MW	LOD (ng/mL)	Ion Charge	Test Compound	MW	LOD (ng/mL)	Ion Charge
SULCONAZOLE	461	5	+	TERBUTALINE	274	2	+
ATROPINE	289	5	+	WARFARIN	308	5	–
ESTRADIOL	272	1	+	PIROXICAM	331	2	–
PYRIMETHAMINE	249	2	+	CHLORTHALIDONE	339	10	–
DARICON(R)	381	5	+	PROGLUMIDE (NA SALT)	356	5	–
CIMETIDINE	252	5	+	CHOLICACID	409	5*	+
SOTALOL HCL	309	5	+	4-PHENYLPIPERIDINE	161	10*	+
IMODIUM(R)	514	0.5	+	CORTISOL	362	2*	+
LEVASOLE(R)	241	1	+	PROPRANOLOL	259	5	+
VERAPAMIL	454	0.5	+	MIDAZOLAM	325	1	+
QUINIDINE	324	5	+	TERFENADINE	471	0.5	+
ATENOLOL	266	20	+	EZETIMIBE	409	10	+
CAFFEINE	194	5	+	SN-38	392	10**	+
FINLEPSIN	236	10	+	HYDROXYMIDAZOLAM	341	5	+
CLOZAPINE	327	0.5	+	DEXTROMETHORPHAN	271	5	+
HYDROXYZINE DIHYDROCHLORIDE	448	2	+	BUFURALOL	298	0.5	+
LABETALOL HCL	365	2	+	HYDROXYTESTOSTERONE	304	NS	+
LOMEFLOXACIN HCL	388	5	+	OXIDIZED NIFEDIPINE	344	NS	+
LOPERAMIDE HCL	514	0.25	+	RALOXIFENE	474	NS	+
PROPANOLOL HCL	196	2	+				

NS = No signal at 100 ng/mL.
**Added acetonitrile to plasma (2:1).
*Polymide-coated silica tip.

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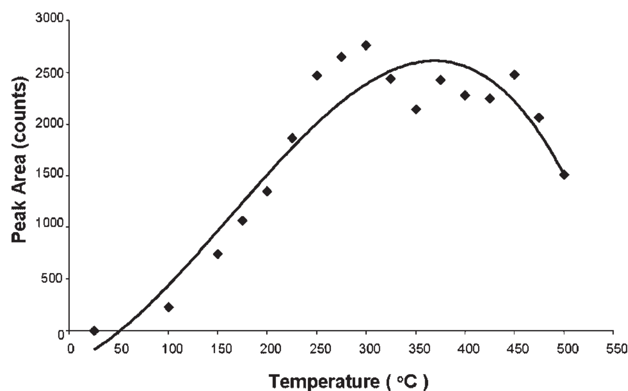

Figure 2. Temperature effect of the gas heater on sensitivity of DART-MS/MS (Roche compound A, 100 ng/mL in dog plasma).

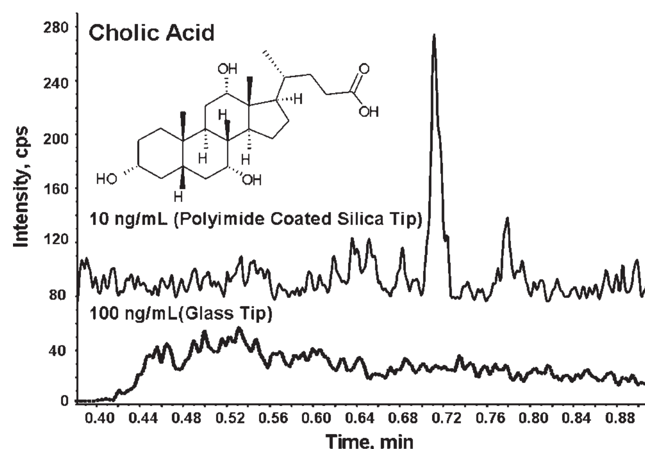

Figure 4. Influence of different probe materials on sensitivity of a carboxylic acid compound.

result obstruct the analyte molecules entering into the gas phase from the plasma sample bulk. An optimal temperature can be found from a bell-shaped signal vs. temperature plot, as seen in Fig. 2. The signal and temperature relationship was found to be virtually compound-independent based on the compounds tested in this study. The temperature selected in this study was 300°C. Gas flow also had a limit beyond which the pressure balance of the mass spectrometer would be disturbed and the mass spectrometer would automatically turn off. High gas flow also blew the sample droplets directly into the ceramic tube and mass spectrometer, causing contamination of the system. The selected gas flow in this study was 3.5 L/min.

Other parameters, such as the distance between the ceramic tube and the orifice of the mass spectrometer, the speed of moving the sample probe across the DART source, and the voltages of electrode 1 and the grid electrode, were also tested. They had limited impact, if any, on the assay performance.

The desorption and ionization mechanism is not well established for DART. It is possible that surface interaction of the analyte with the probe plays a role in assay sensitivity. In this study, probes made of regular glass and silica coated with polyimide were both evaluated. Some compounds, typically those with carboxyl groups, would interact with the regular glass surface causing low sensitivity. However, a polyimide coating on the silica appeared to minimize the surface interaction and thereby increase the signal response. Figure 4 shows the sensitivity of cholic acid when different probe materials were used. No signal was detected with a regular glass probe at 100 ng/mL of this test compound, but it was detected with a polyimide-coated silica probe at 10-fold lower concentration.

Besides the differences in the desorption/ionization mechanisms between DART and conventional electrospray ionization (ESI), a major distinction is the amount of material introduced into the ionization source. Compared to 10–100 μL injection volume in conventional ESI, DART works with <1.0 μL. It is a challenge to load more material into a sampling probe with optimal surface chemistry. This is one area that needs more attention and is currently under development.

Reproducibility

As an important aspect of quantitative analysis, reproducibility was evaluated based on repeated injections. Figure 5 shows a typical signal vs. time profile obtained from this system. The reducibility of the analysis of verapamil in either

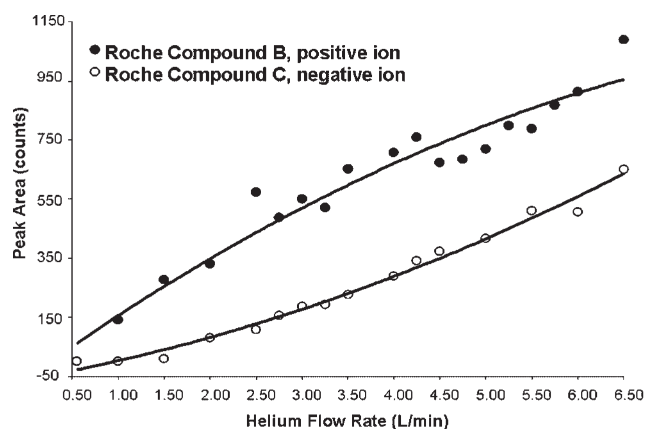

Figure 3. Effect of helium flow rate on sensitivity of DART-MS/MS (100 ng/mL in dog plasma).

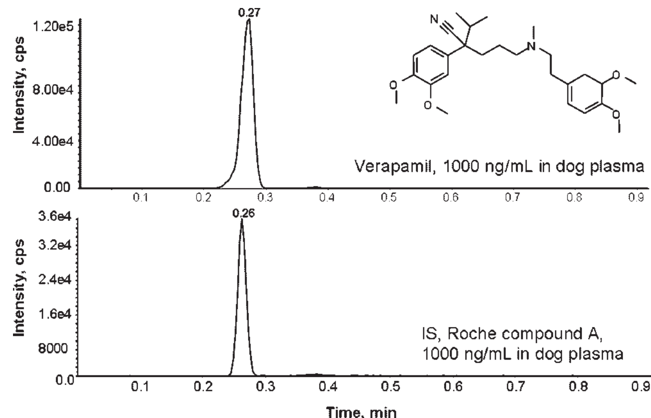

Figure 5. A typical profile of quantification analysis with DART-MS/MS.

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a neat solution of water/acetonitrile (50:50) or dog plasma has been tested with the DART-MS/MS system. No internal standard was used in the experiment. Interestingly, the reproducibility of plasma samples was much better than that of neat solutions. The %CV of a neat solution at 1 μg/mL based on eight injections was 21.7%, while for plasma samples the %CV was 5.86% based on eleven injections. It is possible that sample viscosity influences the reproducibility in this probe-based sample presentation. During sample evaporation and adsorption, it is speculated that the shape/size of the sample liquid film coated on the probe (which contribute to signal response) constantly change as a result of gravity. A high viscosity sample, less affected by gravity, tends to maintain better consistency in liquid shape/size between samples and therefore is associated with lower variability. It was not necessary to use an internal standard for plasma analysis because the possibility of sample preparation or instrument variability is very low due to the simple and fast process. The precision test for verapamil in plasma with and without the internal standard (IS) (not a radiolabeled compound of the analyte) has been carried out; the data indicated that the IS did not improve the precision of plasma sample analysis. However, an IS should be helpful if it is a radiolabeled compound of the test compound or under certain circumstances such as samples containing significant amounts of organic solvent.

Matrix effect

Matrix effect, of which ion suppression is a large contributor, is a major disadvantage of the ESI process. In DART this effect was also observed in plasma samples when compared to a neat solution. A difference in sensitivity of verapamil in a neat solution vs. in dog plasma was observed. The peak height of the analyte with an identical concentration in the neat solution was approximately 20-fold higher than in the plasma sample. Two tests were performed to understand the matrix effect. The results are given in Fig. 6. First, 100 ng/mL verapamil in dog plasma was diluted with water to 50, 35, 25 and 20 ng/mL. The signal response decreased with concentration due to dilution. However, the decrease was not proportional to the concentration decease due to the accompanying reduction in matrix effect. The signal only decreased 26% and 38% when the concentrations decreased to 50% and 20%. In the second test, the same amount of verapmail (fixed final concentration 100 ng/mL) was spiked into plasma/water at varying % water (ranging from 0–80%). Sensitivity significantly increased 3.5-fold with an increase in water content up to 80%, suggesting the presence of a matrix effect and a way of reducing it based on dilution with water. This result indicated that the matrix effect on DART was lower and the sensitivity was higher for clean samples, the same as an ESI source. Figure 7 shows the data from the test of the sensitivity for verapamil in solutions with different volatility and viscosity and similar matrix composition. This result proved that high volatility and low viscosity are good for the sensitivity of DART. As we mentioned before, low volatility and high viscosity are favorable for reproducibility, but unfavorable for the sensitivity. Therefore, a clean sample with appropriate volatility and viscosity will provide high-quality data in DART analysis.

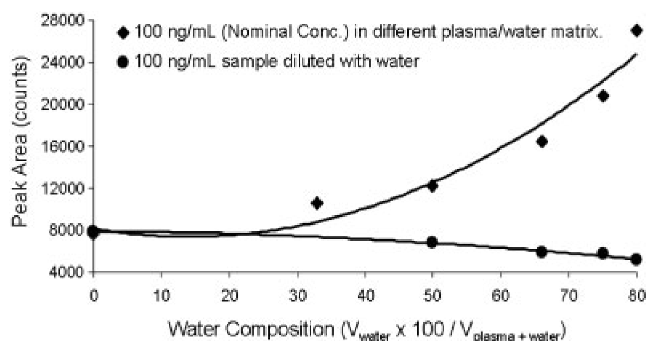

Figure 6. Matrix effect of DART for analysis of verapamil in samples by varying water content.

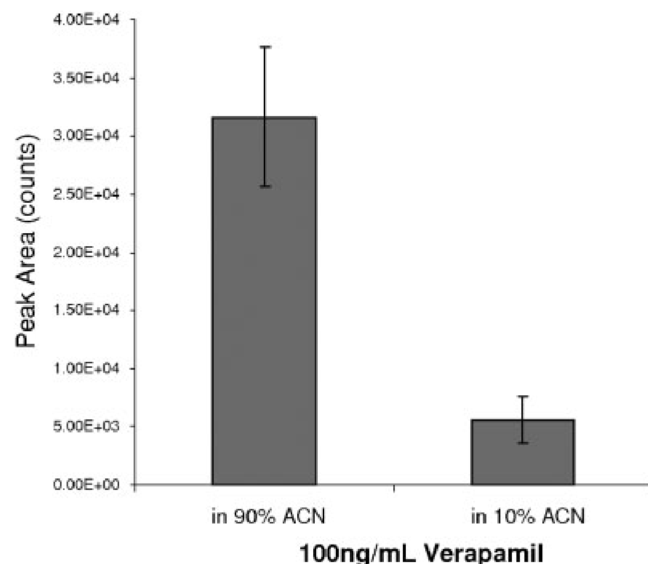

Figure 7. Matrix effect of DART for analysis of verapamil in samples with different volatility (n = 6).

Measurement of drug concentration in the brain is necessary in the drug development of central nervous system (CNS) disorders. Ideally, it is desirable to quantify brain drug level based on a plasma calibration curve to reduce workload (plasma is always measured anyway). This would require the matrix effect being the same between the two matrices. However, it is common to see more significant brain matrix effect than plasma in conventional LC/MS/MS under ESI or even APCI. To investigate whether DART would offer any benefit in this matrix compatibility, we evaluated three CNS compounds based on a common calibration curve constructed in mouse plasma against which mouse brain samples (same concentration levels as plasma calibrants) were measured. The data are given in Fig. 8. The individually back-calculated brain values displayed noticeable variability, but the bias from nominal was both positive and negative, indicating a random variability rather than difference between matrices. After regression, the calibration curves of plasma and brain virtually overlapped, indicating a strong similarity in signal response between the two matrices under the experimental conditions.

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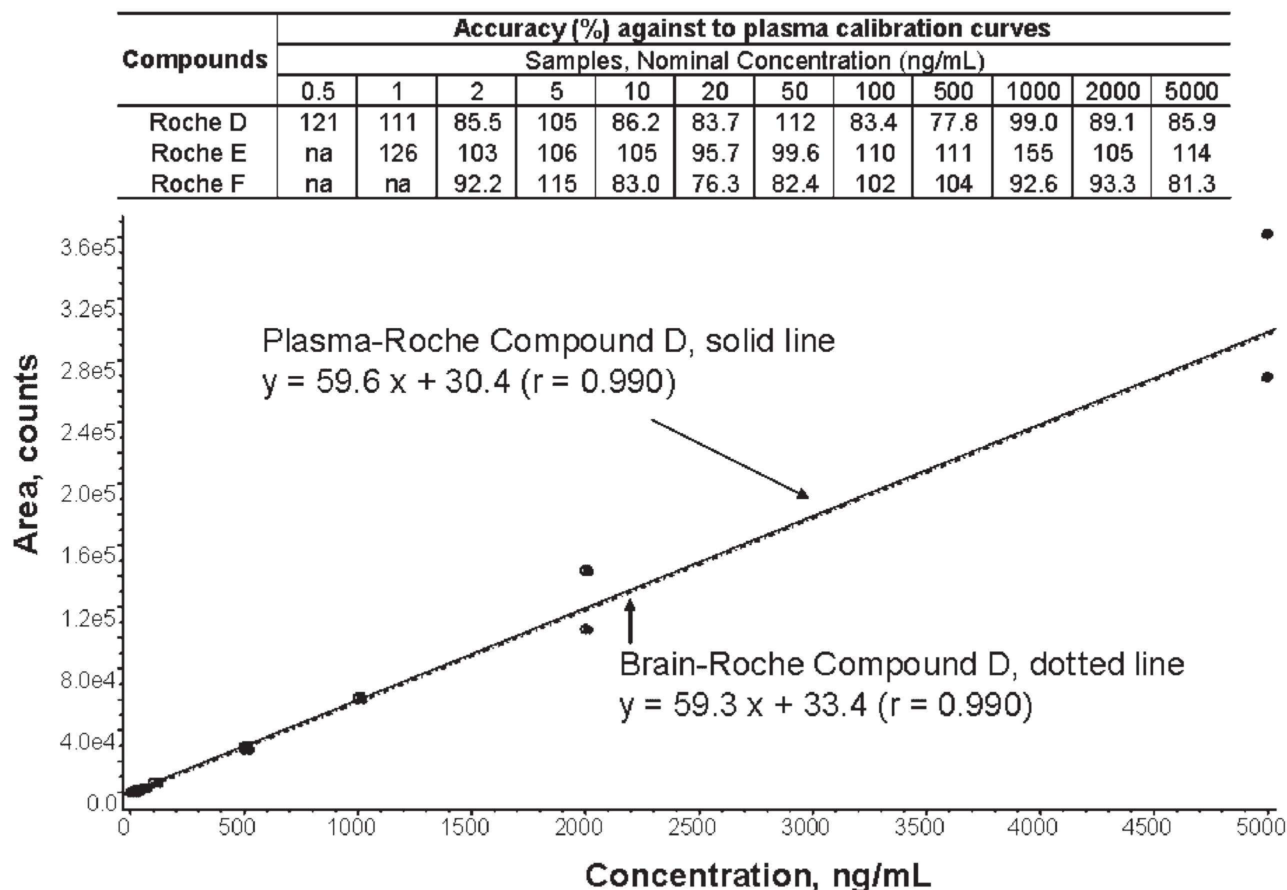

Compounds	Accuracy (%) against to plasma calibration curves											
	Samples, Nominal Concentration (ng/mL)											
	0.5	1	2	5	10	20	50	100	500	1000	2000	5000
Roche D	121	111	85.5	105	86.2	83.7	112	83.4	77.8	99.0	89.1	85.9
Roche E	na	126	103	106	105	95.7	99.6	110	111	155	105	114
Roche F	na	na	92.2	115	83.0	76.3	82.4	102	104	92.6	93.3	81.3

Figure 8. Matrix effect study of DART for analysis of three compounds in mouse plasma samples or brain samples.

Sensitivity and dynamic range

The sensitivity of more than 80 compounds in plasma was tested. The data from commercial compounds are summarized in Table 1. The limit of detection (LOD) of approximately 80% of the commercial compounds was 5 ng/mL or lower. In addition, more than 40 Roche compounds have been tested and 90% of the Roche compounds achieved LODs of 5 ng/mL or lower. Although this result was not as competitive as LC/MS/MS with ESI, the current bench mark, it met the need for most drug discovery and some safety studies.

The quantification dynamic range was evaluated for more than ten compounds. The linear range of these compounds under conventional ESI was between 0.5–5000 ng/mL. The linear range under DART was between 0.5–2000 ng/mL which was similar to ESI. The calibration curves of verapamil, loperamide or bufuralol in dog plasma are 0.5–2000, 0.5–2000 or 1–2000 ng/mL (Table 2).

Ion source degradation

Some metabolites, especially phase II conjugates, can break down in the ionization source converting back into parent compounds and posing positive bias to the analysis. To investigate potential source degradation and its impact, four metabolites were tested including glucuronides of ezetimibe, hydroxybufuralol, dextrorphan, and hydroxymidazolam,

Table 2. Precision and accuracy of DART-MS/MS for the analysis of plasma QC samples

Analyte	Verapamil				Loperamide				Bufuralol			
conc.(ng/mL)	1000	100	2	0.5	1000	100	2	0.5	1000	100	5	1
1	1130	113	1.99	0.339	1100	93.8	2.04	0.511	1160	123	6.00	1.05
2	1080	95.7	1.85	0.395	830	68.0	0.329	0.329	1220	109	4.22	0.966
3	819	106	1.84	0.421	1.840	85.4	1.65	0.542	923	106	3.56	0.911
4	1000	112	1.58	0.424	1090	71.6	1.73	0.413	1010	108	4.04	0.676
5	969	81.6	2.02	0.496	1150	74.4	1.99	0.480	968	102	5.74	0.977
6	918	101	1.80	0.461	1050	65.7	1.79	0.350	1010	112	5.73	1.37
mean	986	102	1.85	0.423	1063	76.5	1.83	0.438	1049	110	4.88	0.99
SD	112	11.8	0.157	0.054	121	10.9	0.152	0.087	116	7.18	1.06	0.225
%CV	11.3	11.6	8.53	12.8	11.4	14.3	8.29	20.0	11.0	6.53	21.7	22.7
%RE	−1.40	1.55	−7.67	−15.5	6.33	−23.5	−8.42	−12.5	4.85	10.0	−2.37	−0.833

Calibration dynamic range for verapami and loperamide was 0.5–2000 ng/mL, for bufuralol was 1–2000 ng/mL.

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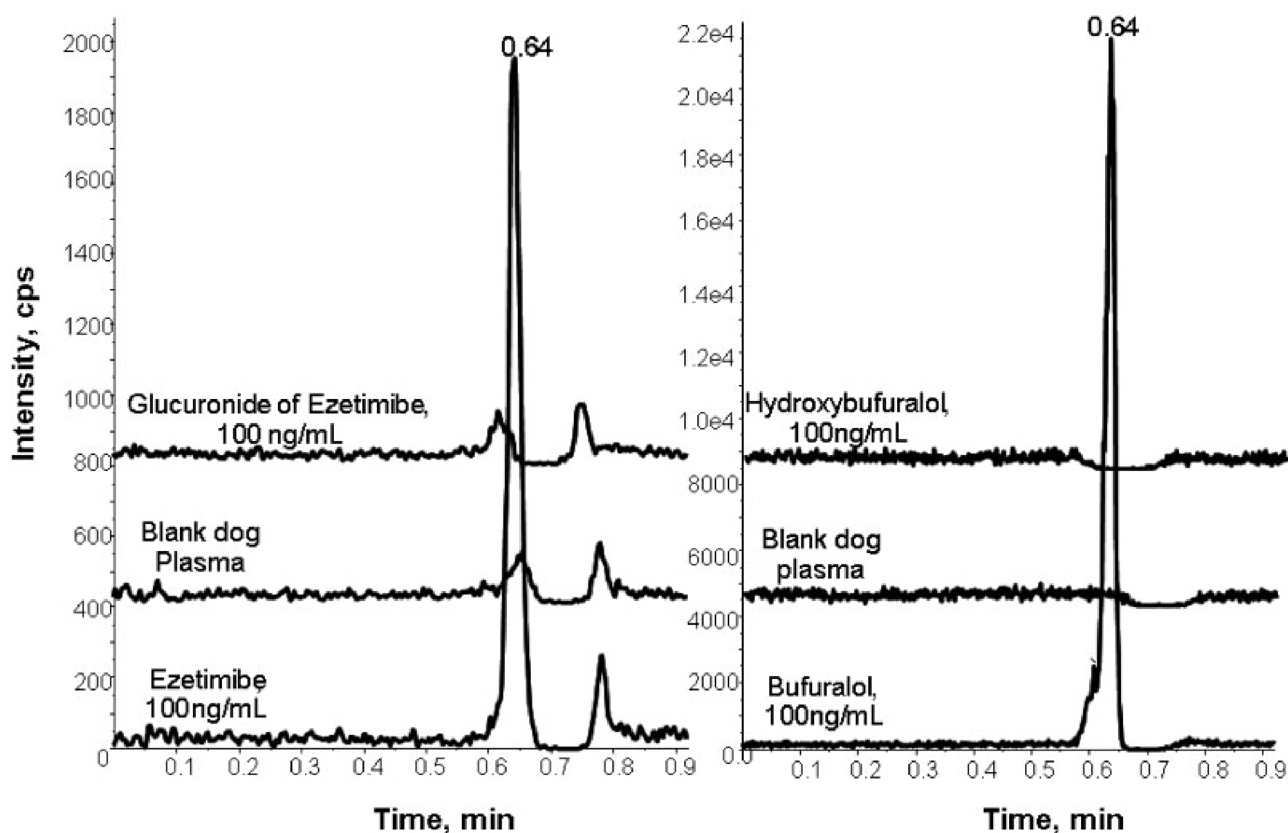

Figure 9. Stability of metabolite compounds in the DART ion source.

representing both phase I and II metabolites. Each compound was analyzed based on three separate injections including metabolite in plasma, parent in plasma and a blank plasma, all using the same MRM transition of the parent compound. In all cases no parent signal was detected from the metabolite sample (Fig. 9), indicating either the metabolites tested were stable in the DART source or any degradation, if it occurred, did not interfere with the analysis of the parent compounds.

Accuracy and precision

Despite many applications of DART in qualitative analysis, quantitative analysis, especially in biological matrices, has not been sufficiently addressed in the literature. In this study after systematic optimization of experimental conditions, verapamil, bufuralol and loperamide were evaluated for their performance in quantitation. These compounds were spiked into dog plasma and analyzed based on QC samples

Table 3. Comparison of pharmacokinetic (PK) parameters obtained from LC/MS/MS and DART-MS/MS

		Loperamide						Verapamil		
		IV			PO			IV		
PK Parameter	Unit	DART	ESI	Diff%	DART	ESI	Diff%	DART	ESI	Diff%
Dose	mg/kg	0.5	0.5		2.0	2.0		0.5	0.5	
$t_{1/2}$	h	1.83	1.66	10.6	10.5	11.1	−5.02	2.77	2.78	−0.3
T_{max}	h	0.08	0.08	0	1.00	1.00	0	0.08	0.08	0
C_{max}	ng/mL	76.2	83.9	−9.21	4.88	5.79	−15.7	63.3	56.1	12.9
AUC_{last}	h*ng/mL	142	158	−9.97	30.0	33.4	−10.1	88.0	84.6	3.94
AUC_{all}	h*ng/mL	158	171	−7.46	53.9	60.9	−11.6	107	99.8	7.27
AUC_{inf}	h*ng/mL	147	162	−8.85	75.2	88.3	−14.8	97.5	92.9	5.71
Vz	mL/kg	8967	7389	21.4				20483	21720	−5.69
CL	mL/h/kg	3391	3091	9.71				5129	5421	−5.40
Vz/F	mL/kg				0.40	0.36	11.5			
CL/F	mL/h/kg				0.03	0.02	17.2			
MRT_{last}	h	1.85	1.67	10.6	3.7	3.7	0.61	1.93	1.82	6.46
MRT_{inf}	h	2.17	1.89	15.0	15.4	16.3	−5.52	2.91	2.65	9.86
%F	%				25.5	27.3	−6.55			

ESI: ElectroSpray Ionization (LC/ESI/MS/MS).

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consisting of four concentration levels and calibration curves. The accuracy and precision were assessed and data are summarized in Table 2. The data indicated that the precision and accuracy meet the acceptance criteria for a quantification analysis to support discovery studies. The DART-MS/MS system should be able to be applied to quantitative analysis for biological samples.

Application of DART-MS/MS

DART-MS/MS has been used for significant numbers of pharmacokinetic and metabolism studies. The results from DART-MS/MS have been compared with the data obtained from LC/MS/MS. Table 3 shows some of these data. A comparison of the metabolic stability data obtained by DART-MS/MS and LC/MS/MS has been done. The study results indicated that the pharmacokinetic parameters and profiles from DART-MS/MS and LC/MS/MS were very similar.

CONCLUSIONS

The DART-MS/MS system has demonstrated some advantages, for example, no sample preparation and LC separation, over conventional LC/MS/MS in drug research; especially in bioanalytical support of discovery and early development programs where simplicity and operating speed are a premium. The eradication of sample extraction and LC separation allowed rapid turnover. The analysis time for a sample with DART-MS/MS is about 54 s, but with conventional LC/MS/MS is from 3–5 min. However, the instrument run time currently is limited by the cycle time (35–45 s) of the autosampler designed for conventional LC/MS/MS. A new probe arrangement (four tips) is currently under development aiming at much speedier sample introduction in order to be compatible with the DART scan time (5–10 s). This new probe will allow four samples to be analyzed in 54 s. The capital investment is low because the ion source can be installed onto most existing MS/MS systems. An added environmental benefit is the elimination of the use of LC mobile phase. Some common difficulties associated with LC/MS/MS such as carryover, contamination and peak shape integrity are also removed. DART-MS/MS is less sensitive than LC/MS/MS and has difficulty in solving the matrix effect for some special samples at this time. However, since DART-MS/MS is new, this is the first publication about its application of quantitative analysis for biological samples, these problems will be overcome when this technology is improved. DART-MS/MS is a novel system still under development. An in-depth study needs to be performed to understand why some compounds cannot be ionized in DART. In addition, sensitivity needs to be improved for more general use in late-stage drug development.

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