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Assessment of paper spray ionization for quantitation of pharmaceuticals in blood spots☆

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ABSTRACT

Paper spray uses solvent electrospray to produce gas phase ions from samples deposited on paper or other porous media by applying a high voltage to the wet substrate. The method is amenable to the analysis of small and large molecules and is applied here to pharmaceuticals from dried blood spots, making it potentially useful for quantitation of drugs during clinical trials or for therapeutic drug monitoring in a hospital or clinic. Several topics related to the analytical characteristics of paper spray for analyzing drugs in dried blood spots are explored. (1) The effect of protein–drug interactions was studied by comparing the ratio of propranolol (PRN) and atenolol (ATN), two drugs with widely disparate protein binding properties, in water and in blood and by spiking a deuterated analog of PRN into blood already containing PRN and then allowing the blood to incubate for different lengths of time. In the first experiment, the ratio of PRN to ATN was the same in both water and blood. In the second experiment, the ratio of PRN and PRN d7 was the same regardless of how long the deuterated analog was allowed to equilibrate in the blood. These results suggest that protein binding does not affect analyte signal, and that paper spray therefore measures the total drug concentration in blood at least in this case. (2) Alternative methods for incorporation of the isotopically labeled internal standard (IS), which is needed for quantitative results when analyzing drugs in dried blood spots, were tested. Because it is often not feasible to add the standard to the liquid blood at the point of collection, the IS must be incorporated into the dried blood in some way. The IS PRN d7 was combined with dried blood spots containing PRN in three different ways: by pretreating the paper with an IS solution, by doping the IS into the spray solvent, and by adding an IS solution to a punched out section of a DBS. The variance of the method was less than 8% when the IS was added to the paper either before adding the blood or when adding it afterwards to the dried blood punch. The variance was 16% when the IS was added to the solvent eluent. In all cases, the recovery of PRN was lower than that of the IS. (3) Finally, matrix effects were assessed by measuring the signal obtained for PRN d7 in matrices ranging from neat solution, to blank blood, to blood containing 1 μg/mL of PRN. A neat solution was found to give approximately 10 times higher signal than blood, indicating that suppression of ionization is occurring. The presence of a high concentration of PRN did not affect the response for PRN d7.

1. Introduction

Electrospray ionization (ESI) mass spectrometry, developed by John Fenn and his co-workers beginning in the early 70 s, has been a transformative technology. Many major barriers to taking full advantage of mass spectrometry have been overcome by the ability to produce gas phase ions of fragile species from aqueous solution. Fields such as proteomics and pharmaceutical research and development have benefited from the ability to perform MS analysis on large biomolecules and from the improved coupling of MS to high performance liquid chromatography (HPLC) that grew out of Fenn's work on ESI. But while ESI has been used routinely in mass spectrometry for almost two decades, variations and exten-

☆ In appreciation of the scientific contributions of John Fenn and in admiration of his personal qualities.

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sions to the technique continue to emerge. New methods that make use of the electrospray phenomenon have emerged in recent years, including desorption electrospray ionization (DESI), which uses a pneumatically assisted electrospray to analyze surfaces in the ambient environment [1], electrospray assisted laser desorption/ionization (ELDI), which uses an electrospray plume to ionize the neutrals thermally desorbed from a surface using laser ablation [2], and the utilization of ESI electrochemical processes to drive analytically useful oxidation/reduction reactions [3]. The first of those two methods, DESI and ELDI, are part of a growing family of ambient ionization methods that can be used to analyze samples in the open air without sample preparation [4]. These new approaches have continued to expand the range of analytes and samples that can be interrogated by mass spectrometry.

Another recently described variant of electrospray ionization is paper spray ionization [5,6], in which samples are deposited onto paper; after application of solvent and a high voltage, analyte transport and ionization by the electrospray process is affected. Fenn himself touched on this idea over a decade ago when he filed for a patent for performing ESI through a wicking element [7], which he saw as a way to couple the different fluid flow regimes of ESI and HPLC. He also mentioned the possibility of coupling paper chromatography or thin layer chromatography (TLC) to mass spectrometry by performing electrospray ionization directly from the chamfered tip of the paper or TLC plate. We have taken this idea further by using the paper as a self-contained apparatus combining sample storage, extraction, crude separation if desired, analyte derivatization if required, and ionization.

The development of this method has been driven primarily by the need for faster and simpler procedures for the analysis of pharmaceuticals from whole blood. Tests for chemicals found in blood are typically carried out on a liquid sample, usually serum or plasma isolated from whole blood. The required storage, transportation, and handling of liquid blood or blood components present challenges. While blood in liquid form is essential for some tests, others can be performed on blood or other biofluids that have been spotted onto a surface (typically paper) and allowed to dry. Dried blood spot (DBS) collection for subsequent chemical analysis was first introduced in the 1960s for phenylketonuria screening using a bacterial inhibition assay that detected elevated levels of phenylalanine in blood dried on a piece of filter paper [8]. Currently, dried blood spot (DBS) analysis combined with mass spectrometry has become a standard technique for neonatal screening of inherited disorders of metabolism involving amino acids, fatty acids, and organic acids [9–11] using relatively cumbersome (though reliable) methods to extract the analytes of interest from the dried blood. Recently, increased attention has been given to DBS due to the reduced blood volume needed [12], the increased stability of drugs and drug metabolites in dried blood compared with liquid blood [12,13], and because the storage and shipment of dried blood cards is more convenient since there is no need for a freezer or dry ice, and the biohazard problems are less severe [14]. In addition to the accepted methods for DBS analysis using liquid extraction followed by direct injection–MS or HPLC–MS, other techniques have been introduced recently, including direct analysis of the DBS by DESI [15] and automated extraction via a TLC–MS interface device [16,17].

Preliminary experiments indicated that paper spray has the potential for rapid quantitative analysis of drugs from whole blood. The chemotherapeutic drug imatinib, for example, was detected as low as 50 ng/mL from dried blood, well below the typical therapeutic value for this drug [18], and the analysis showed good linearity over the entire range examined (62.5 ng/mL to 4 μg/mL) [5]. The paper spray method is also simple (it involves a single step), it can be performed on less than a microliter of blood, requires small amounts of organic solvent, and uses low cost and readily available materials. These characteristics make paper spray, implemented using a disposable cartridge coupled to a fully automated, low cost mass spectrometer, potentially useful in a clinic or hospital for therapeutic drug monitoring.

This report addresses several important issues with respect to the analysis of drugs by paper spray, namely the role of protein–drug interactions, incorporation of an internal standard (IS) for quantitation, and means of controlling the amount of blood analyzed.

2. Experimental

2.1. Materials

Propranolol (PRN) was purchased from Sigma-Aldrich (St. Louis, MO), and PRN d7 was acquired from CDN isotopes (Pointe-Claire, Quebec, Canada). Ahlstrom 226 paper was obtained as a sample from ID Biological Systems (Greenville, SC). Whatman Grade 1 chromatography paper was purchased from VWR. Bovine blood (sodium citrate) was purchased from Innovative Research (Novi, MI).

2.2. Mass spectrometry and paper spray ionization

All experiments were performed using a TSQ Quantum Access Max (Thermo Scientific, San Jose, CA) in the selected reaction monitoring (SRM) mode, sometimes known as multiple reaction monitoring (MRM), which detects specific product ions produced by collision induced dissociation (CID). The SRM parameters were as follows: PRN: m/z 260 → 183; tube lens: 130 V; Q2 offset (collision energy): 18 V; PRN d7: m/z 267 → 189; tube lens: 130 V; Q2 offset: 18 V; ATN: m/z 267 → 145; tube lens: 123 V; Q2 offset: 27 V.

For paper spray ionization, the paper was cut into a triangle of approximately 10 mm base width × 10 mm height. A schematic of the experiment is shown in Fig. 1A. A volume of solvent, typically 10 μL of 75:25:0.5 methanol:water:acetic acid, was added to the paper using a pipette after the blood spot had thoroughly dried and the spray voltage was then set to 3500 V. Alterations to this general method are noted in subsequent sections.

2.3. Drug–protein interaction

A 50 μL aliquot of an aqueous solution of PRN d7 (20 μg/mL) was added to 950 μL of blood with a PRN concentration of 1 μg/mL. This solution was mixed briefly and incubated at 37 °C for 10 min. An aliquot of the blood (0.5 μL) was then spotted on Whatman Grade 1 chromatography paper for analysis. Next, an identical blood solution was prepared, except that it was mixed quickly and immediately spotted on the paper without allowing significant time for incubation. In both cases, the blood was dried for at least 1 h and followed by addition of solvent and ionization with 10 μL of the 75% methanol with 0.5% acetic acid solution

2.4. Addition of internal standard

Three different methods of adding the IS were investigated: pretreating the paper, doping the IS into the eluent, or adding the IS to a punched out section taken from a dried blood spot. In the first experiment, 1.25 μL of a 2 μg/mL aqueous solution of PRN d7 was added to Ahlstrom grade 226 blood collection paper. The standard was added slowly using a pulled glass capillary to minimize the size of the spot. After the paper had dried thoroughly, 2.5 μL of blood containing 1 μg/mL of PRN was added directly on top of the IS spot using a pipette. The blood spot was allowed to dry and included in a triangular piece of paper which was cut out from the

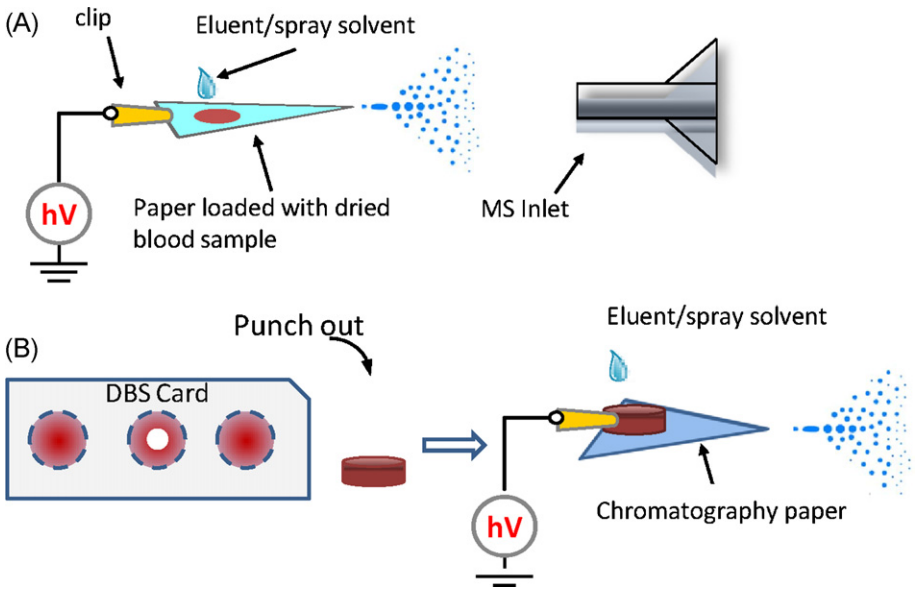

Fig. 1. (A) Schematic of a typical paper spray experiment. (B) Schematic for quantitation from a DBS by taking a punch from the spot and adding an IS solution prior to ionization.

paper. Addition of 20 μL of solvent and application of a high voltage allowed ionization; the larger solvent volume was used in this case because the Ahlstrom 226 paper is thicker and more absorbent than chromatography paper.

In the second experiment of this type, 2 μL of blood containing 1 μg/mL of PRN was added to Whatman Grade 1 chromatography paper using a pipette and allowed to dry. Analyte transport and ionization were performed with 15 μL of 75% methanol with 0.5% acetic acid containing 133 ng/mL PRN d7. The absolute amounts of PRN and PRN d7 were the same.

Finally, an IS solution was added to a punch taken from dried blood spots. Blood aliquots 35 μL in volume and containing 1 μg/mL of PRN were spotted on Ahlstrom 226 paper, forming spots approximately 10 mm in diameter, and allowed to dry for at least 2 h. A punch 7 mm in diameter was taken from the center of the DBS. The blood volume in the punch was estimated to be 17 μL based on the relative area of the punch and the DBS. The same volume and amount of IS was then added by pipetting 17 μL of a 1 μg/mL solution of PRN d7 in 3:1 (v:v) methanol:water onto the blood punch and allowing it to dry. The drug and IS were eluted and ionized by attaching the punch to a paper triangle using an alligator clip and slowly adding 75% methanol with 0.5% acetic acid. A schematic of this experiment is shown in Fig. 1B.

2.5. Ionization suppression

The signal intensity of PRN d7 was compared in several different matrices by examining 0.5 μL of 1 μg/mL PRN d7 in blank blood, in blood containing 1 μg/mL PRN, and in water; each sample was examined using chromatography paper. Ionization was performed as with the previous samples. The average intensity is reported because the intensity was constant within 5% over the course of the experiment and, unlike AUC, the average intensity is independent of the time of analysis.

3. Results

3.1. Paper spray ionization

The general experimental setup for paper spray is shown in Fig. 1A, in which a triangular shaped paper is loaded with a sample, and analytes are transported through the paper and ionized by application of solvent and high voltage. Inspection of the mass spectra obtained by paper spray ionization and visual observation of the Taylor cone and resulting spray plume indicated that ionization likely occurs by the typical ESI mechanism. Mass spectra recorded using a commercial glass nanoelectrospray emitter with a 2 μm tip (PicoTip GlassTip from New Objective) and those acquired by paper spray using identical equimolar solutions of neurotensin and maltoheptaose are shown in Fig. 2. The spectra are qualitatively similar in a number of ways and show similar overall intensity. For both ionization methods, neurotensin is primarily detected as an $[M+2H]^{2+}$ ion, but is also present as $[M+3H]^{3+}$ and in the form of various sodium adducts. Also, for both methods, the peaks corresponding to maltoheptaose are significantly less intense than those of neurotensin, despite their equal concentrations. This is generally held to be due to masking of the more hydrophilic species, maltoheptaose in this case, by the more hydrophobic ones (e.g. neurotensin). This effect becomes less pronounced at smaller droplet sizes [19]. The main difference between the electrospray and the paper spray spectra is the intensity of the sodiated species. The $[M+H+Na]^{2+}$ and the $[M+2Na]^{2+}$ ions of neurotensin and the $[M+Na]^{+}$ ion of maltoheptaose are relatively more intense for paper spray than for nanoelectrospray ionization. This may be due to a higher amount of adventitious sodium or a greater surface area to volume ratio for the paper as compared to the glass electrospray capillary. Another difference between the two is that the baseline noise level for the paper substrate is higher than for the nanoelectrospray source. It is not yet clear what caused this, or if the higher baseline is even a general phenomenon or only occurs under a particular set of experimental conditions.

The onset voltage for paper spray was typically between 2000 and 3000 V, with this value being dependent on solution parameters such as surface tension, as is well-known in ESI [20]. In both positive and negative ion modes, corona discharge could be observed, as indicated by an increase in spray current and a reduction or elimination of analyte signal. In the positive ion mode, this occurred at high spray voltage or if the solvent was not replenished; if the solvent on the paper was allowed to evaporate, a discharge occurred provided the paper was still moist enough to be conductive. In the negative ion mode, it is presumed that the onset voltage for Taylor cone formation is the same as in positive ion

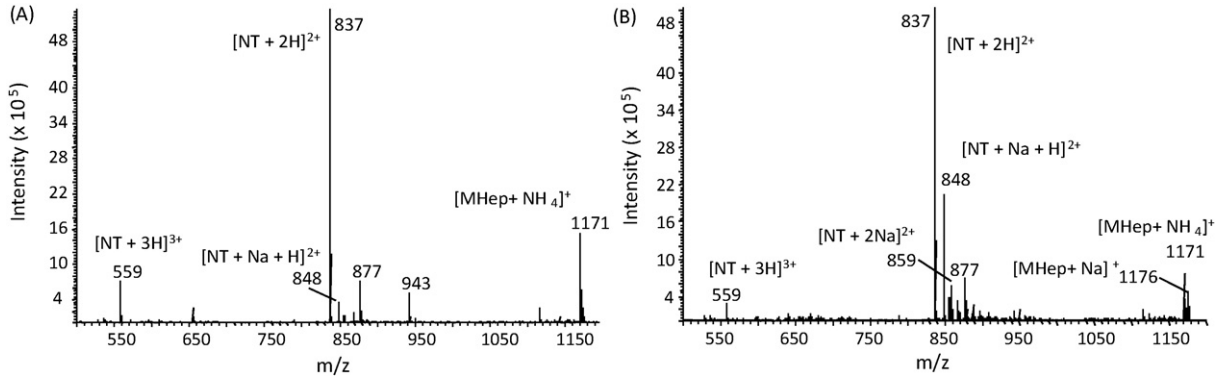

Fig. 2. Full scan positive ion mode mass spectra of 10 μM neurotensin (NT) and maltoheptaose (MHep) in 1:1 methanol:water with 10 mM ammonium acetate collected by (A) nanoelectrospray and (B) paper spray.

mode. However, corona discharge occurred at a much lower voltage than in positive mode. This effect also occurs with conventional ESI [21], but in our hands was more pronounced when using paper as the spray substrate. As such, negative ion analytes could generally only be observed with solvents of low surface tension, such as pure methanol.

During Taylor cone formation on the paper, solvent is replenished at the tip by capillary flow, and excess solvent must therefore be made available to allow wicking of solvent to the tip. Uniform flow to the tip was most conveniently accomplished by depositing a greater amount of solvent onto the paper than could be absorbed. The excess solvent created a pool on the paper or pooled around the alligator clip due to surface tension and flowed to the tip as solvent was depleted. Under these circumstances, the analyte signal was practically constant with respect to time until it abruptly dropped to zero after the solvent was depleted by the spray process and evaporation (Fig. 3A). Alternatively, the paper was either itself in contact with a solvent reservoir or contact was made via a hydrophilic wick. Solvent then traveled through the paper by capillary action analogous to the situation in paper chromatography. Applying 75% methanol:water in this way, the signal from analytes on the paper decayed from an initial maximum until reaching a stable value after about 3 min (Fig. 3B). It is anticipated that using a different solvent system and increasing the size of the paper substrate would lead to well defined elution peaks as a function of time depending on analyte hydrophilicity, again analogous to paper chromatography.

3.2. Drug–protein interaction

Drugs circulating in the blood may be free or bound to plasma proteins, particularly to albumin, alpha-1 acid glycoprotein, and lipoprotein. Careful measurements by equilibrium dialysis or ultrafiltration are generally required to measure the free drug fraction to avoid disrupting the drug–protein interactions [22], while solid–liquid or liquid–liquid extraction is used to recover the total amount of drug (free plus bound). It seems likely that paper spray, which involves first drying the blood sample followed by essentially an extraction using a polar organic solvent, would measure the total drug concentration. This is because the plasma proteins are probably denatured during the drying process, and noncovalent interactions are likely to be further disrupted by the addition of organic solvent. We performed two simple experiments to confirm this hypothesis. The first experiment employed the drugs PRN and ATN, which have significantly different protein binding characteristics: PRN is about 80% bound at the chosen concentration (1 μg/mL) [23] while ATN exhibits negligible plasma protein binding [24]. The full scan mass spectrum of 500 ng of PRN and ATN analyzed from a neat solution together with the MS/MS spectra of the two drugs are shown in Fig. 4. To determine the effect of protein binding, the ratio of these two drugs was compared when analyzed from neat solution and when analyzed from blood. As shown in Table 1, the ratio of the area under the curve (AUC) for PRN to ATN was not significantly different ($P > 0.05$) in pure solution versus in a blood matrix, implying that the degree of protein binding does not affect analyte detection.

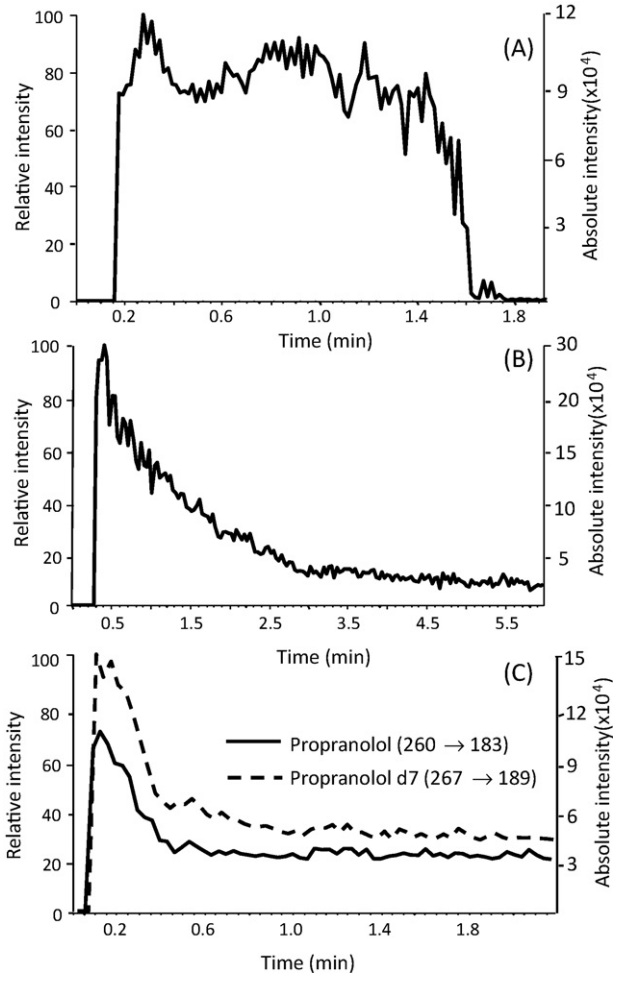

Fig. 3. Ion chronograms (signal versus time) for PRN, 500 ng deposited on paper and eluted by either (A) depositing 10 μL of solvent all at once or (B) allowing solvent to wick through the paper continually (C) for PRN and PRN d7 for the addition of the IS solution to the DBS punch, followed by the elution with solvent.

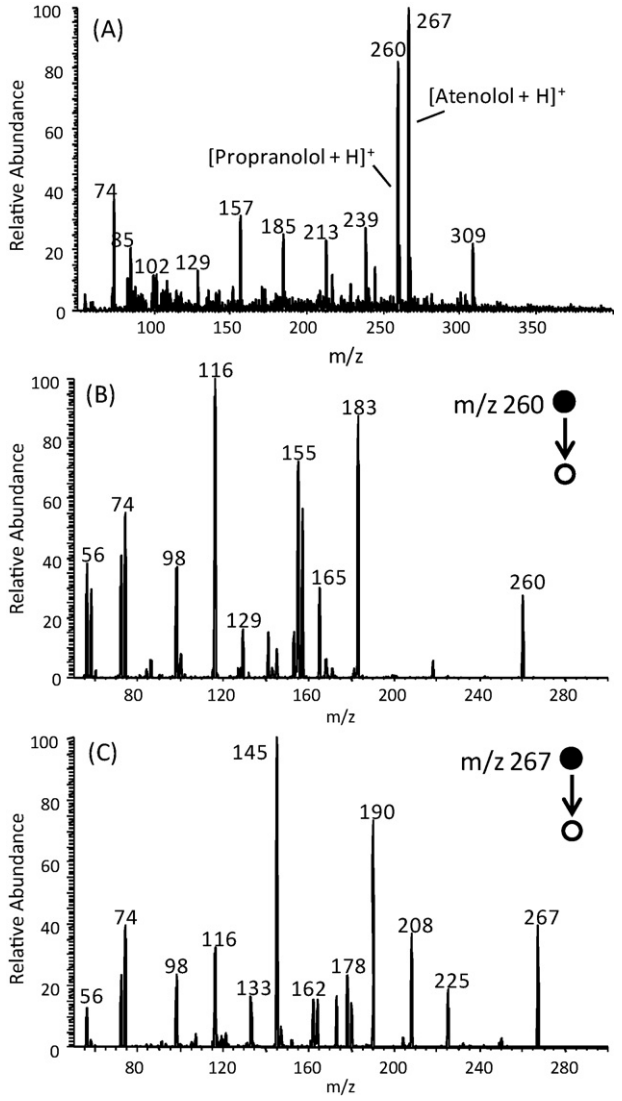

Fig. 4. (A) Full scan mass spectrum of mixture of 500 ng each of PRN and ATN analyzed by paper spray. (B and C) Tandem mass spectra of PRN and ATN, respectively.

To explore further the possible effects of protein binding, a second experiment was performed in which the d7 form of PRN was spiked into a blood sample at 4 °C that already contained an equal amount of PRN. The blood sample was then immediately deposited on the paper and dried, or it was warmed to 37 °C and incubated for 10 min. In the latter case, protein binding to the internal standard should occur, while very little equilibration should take place in the former. The ratio of the area under the curve (AUC) between PRN and PRN d7 was not significantly different between the two cases ($P > 0.05$), again indicating that the degree of protein binding in the blood does not affect the detection of the drug. Taken together, these results suggest that paper spray measures the total amount of drug in the dried blood spot in this case and by inference based on the selection of the drugs tested, more generally.

3.3. Addition of internal standard

Quantitation by mass spectrometry is typically performed with an internal standard, ideally isotopically labeled, because the presence of the standard can overcome matrix effects and other sources of irreproducibility that are inevitably introduced during extraction and analysis [25]. One of the analytical challenges presented by the use of DBS rather than liquid plasma or blood is the addition of the IS. Whereas the IS can be thoroughly mixed into a liquid sample prior to extraction, this is not possible with a dried blood spot. In normal analytical procedures for DBS analysis, a punch of known size is taken from the DBS and extracted with a volume of solvent containing the IS [12]. For paper spray, the following methods for adding the internal standard to the DBS were explored: (i) treating the paper with the IS prior to spotting the blood, (ii) spiking the internal standard into the eluent/spray solvent, and (iii) adding an IS solution to a dried blood punch taken after the blood was thoroughly dried.

In the first experiment, the working solution of the internal standard was deposited onto the paper and allowed to dry. A volume of blood containing PRN was then spotted such that the blood completely covered and extended beyond the area wetted by the IS; the absolute amounts of PRN and PRN d7 on the paper were the same. The average and standard deviation of the PRN:PRN d7 obtained from 7 trials is shown in Table 2. When adding the internal standard in this way, the precision was worse than when the IS was mixed in the liquid blood (8 versus 3%). A variance of 8% is still acceptable for most applications, being less than the 15% cutoff specified by the Food and Drug Administration for bioanalytical method validation.

Interestingly, the recovery of PRN, which was present in the blood, was evidently significantly lower than for the IS, which was spotted on the paper prior to blood spotting; the ratio of PRN to PRN d7 was 0.72 while a ratio of about 1.0 was expected based on control experiments where the drug and the deuterated analog were mixed together in the liquid blood. The experiments described in section 3.2 indicate that this difference is most likely not due to drug–protein interactions. An alternative explanation is that the PRN, but not the PRN d7, is fully embedded within the dried blood matrix. The PRN may not be recovered from this matrix as efficiently because the solvent does not fully wet and penetrate the matrix of dried blood or because of hydrophobic interactions between the drug and the components of the dried blood. Another possible explanation is that there are small, local variation in how the blood is absorbed by the paper and that there were therefore pathways through the paper that did not contain as much blood. The solvent would likely preferentially take these paths because the contact angle of the solvent with the substrate would be lower if less or no blood was present.

Another approach taken to adding the internal standard to the DBS was to incorporate the IS into the solution used to extract and ionize the drug from the dried blood. In this experiment, PRN dissolved in blood was deposited on the paper and allowed to dry. A spray solvent containing the same absolute amount of PRN d7

Table 1
Effect of protein–drug interactions on analyte detection.

PRN:PRN d7 (AUC, $N = 7$)	PRN d7 incubated in blood with PRN prior to analysis		PRN d7 spiked into blood with PRN and spotted immediately	
	Mean: 1.11	S: 0.03	Mean: 1.09	S: 0.03
PRN:ATN (AUC, $N = 7$)	PRN and ATN in blood		PRN and ATN, neat solution	
	Mean: 0.76	S: 0.06	Mean: 0.81	S: 0.08

Table 2
Drug recovery and precision for various methods of internal standard addition.

Method of IS addition	Mean PRN/PRN d7 (AUC, $N = 7$)	Standard deviation	Relative standard deviation (%)
Mixed in liquid blood	1.05	0.03	3
Dried on paper prior to blood spotting	0.73	0.06	8
Added to dried blood punch[a]	0.72	0.04	6
Present in spray solvent	0.26	0.04	16

[a] $N = 6$. One value rejected by Q test at 95% confidence.

Table 3
Ionization suppression effects.

	Mean signal intensity	Standard deviation	RSD (%)
PRN d7 in blood containing 1 μg/mL PRN	1.0×10^4	1×10^3	16
1 ppm PRN d7 in blood	1.1×10^4	2×10^3	13
1 ppm PRN d7 in water	2.1×10^5	3×10^4	12

was then used for analysis. As shown in Table 2, the ratio of PRN to PRN d7 was significantly lower than in the first experiment at 0.26 versus 0.72. This was presumably caused by the slow recovery of PRN from the paper surface, evidence for which is provided by the fact that analytes dried on the paper could be detected even after several applications of spray solvent. PRN d7, on the other hand, was already fully dissolved in the spray solvent and was therefore detected with greater intensity. Also, the precision of this method was significantly worse than the previous experiment at 16%. The additional sources of irreproducibility were likely introduced into the experiment due to variation in how the solvent was applied and its distribution on the paper, which affected the extraction of PRN from the dried blood spot.

In the experiments described thus far, a measured volume of blood was deposited onto the paper using a pipette. Given the ultimate goal of designing a disposable cartridge to perform the full analysis automatically, a calibrated capillary could be used to draw up a reproducible volume of blood by capillary action from a heel or finger stick and subsequently deposit it onto the paper. Another possible approach, explored here, is arguably simpler and more analogous to the current DBS method: taking a punch of reproducible size from the DBS. The amount of blood is controlled because the paper, which consists of tightly controlled cotton fibers, absorbs the blood evenly and reproducibly such that identically sized punches should contain identical amounts of blood regardless of the volume of blood deposited on the paper. This method is attractive for the design of a disposable cartridge because of its simplicity. In this experiment, depicted in Fig. 1B, a punch was taken from a dried blood spot and a solution containing the internal standard (PRN d7 in this case) was added to the punched out section and allowed to dry. The punched out disc was then placed on top of another piece of paper cut in a shape amenable to paper spray, and solvent was allowed to flow through the disc and onto the paper. The drug and the IS are extracted from the punch and transferred to the spray substrate by the solvent and ionized.

The result of this experiment is shown, together with those of the previous two types of IS addition methods, in Table 2. The variance obtained for this experiment was similar to that found when the internal standard was deposited first followed by the addition a controlled volume of blood (6 versus 8%). This indicates that controlling the blood amount by taking a blood punch rather than by spotting a measured volume of blood did not introduce additional sources of irreproducibility. It is recognized, however, that this experiment represented an ideal case. The blood was carefully, though manually, deposited on the paper, and the blood used in each of the replicate measurements was identical. In a real setting, variations in the blood hematocrit, the volume of blood deposited on the paper, and minor inconsistencies in the blood collection procedure will almost certainly lead to greater variability.

The ratio of PRN to PRN d7 obtained for this experiment was also similar to the case where the internal standard was deposited first followed by the addition a controlled volume of blood. It should be noted that the amount of blood present on the punch was not known exactly but was instead estimated based on the area of the blood punch compared to the area of the entire blood spot. Nevertheless, it is reasonable to conclude that the recovery of PRN, which was dissolved in the blood, was lower than for the PRN d7, which was added to the dried blood punch.

The ion chronograms recorded for PRN and PRN d7 during a typical experiment are shown in Fig. 3C. The intensity dropped off from an initial intense maximum after about 10 s and then remained stable for over 10 min, provided the solvent was steadily replenished. The long duration of the signal provides ample time for signal averaging, monitoring multiple reaction channels in the case of a triple quadrupole mass spectrometer, or performing MS^n in the case of an ion trap. It also indicates that there is significant opportunity for improvement of the method's sensitivity by eluting the analyte faster or by preconcentrating it prior to ionization.

3.4. Ionization suppression

Ionization suppression, in which other components present in the matrix reduce the ionization efficiency of the analyte, is known to occur with ESI. This effect was studied for paper spray by comparing the average signal intensity obtained for PRN d7 in three different matrices: water, blood, and blood containing 1 μg/mL of PRN (Table 3). The average signal intensity of PRN d7 in blood was the same whether PRN was present or not, indicating that PRN does not suppress the internal standard response at these concentrations. It is likely that such suppression will occur, however, at very low concentrations of drug where the internal standard is several orders of magnitude more concentrated. Further experiments are needed to determine the linear dynamic range, although previous studies with paper spray indicate that the method is linear over at least three decades [5].

When spotted in water, the average response of PRN d7 was about 10 times higher than when spotted in blood. The lower signal when analyzed from blood is likely actually a combination of ionization suppression and poorer recovery of the analyte and IS from the dried blood matrix. The presence of an internal standard at a constant concentration can largely nullify ion suppression as a source of variability, but the limit of detection of the method still suffers.

4. Conclusions

We have made an initial assessment of the strengths and weaknesses of several procedures for implementing paper spray

ionization for the quantitation of small molecule pharmaceuticals from dried blood spots. The degree of protein-drug interactions was not found to affect analyte availability, and acceptable precision was attainable when the internal standard was applied either by pretreating the paper prior to blood collection or by adding an internal standard solution after sample drying. Ionization suppression was found to occur as indicated by the lower analyte signal in blood relative to a clean matrix. Additional work is needed to establish the utility of this method for quantitation. Namely, only two compounds were studied in the present work, which is inadequate to establish the general applicability of paper spray. Also, a study using real blood samples from drug doped animals is needed.

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