Development of a paper spray mass spectrometry cartri

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Overview

- A novel paper spray cartridge performs extraction, preconcentration, and sample ionization.
- Selective enrichment of target molecules from larger sample volumes and removal of the matrix from complex samples such as plasma.
- Significantly improved the signal intensity for the analysis drugs in plasma samples.
- Improved the detection limits significantly by a factor of 14 to 70 depending on the drug tested.
- Quantitative analysis: a linear calibration curve for the drugs from the LOD to 1 µg/mL.

Introduction

- Paper spray mass spectrometry is a method for performing rapid, direct analysis of samples spotted on paper or another porous substrate.
- Targeted quantitative analysis of drugs and drug metabolites directly from dried biofluids.
- Paper spray simplify and expand the utility of mass spectrometric assays.
- Detection limits of paper spray analyses from complex samples are sometimes inadequate.
- Simply increasing the sample amount does not significantly increase signal intensity or improve detection limits for direct paper spray analysis.
- Improving detection limits requires matrix removal and/or concentration of the analyte.

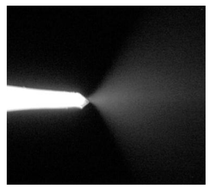


Figure 1. Picture of cone-jet generated from paper

Methods

- The cartridges were made from Delrin® plastic on a milling machine.
- MS analysis was performed using on Thermo Fisher LTQ-XL mass spectrometer.
- The cartridge consisted of two parts, a bottom part (LWH: 40mm x 26mm x 6mm) and a top part (LWH: 14mm x 22mm x 13mm) joined together using a tongue and groove.
- The SPE material was a 1:1 mixture of Select HLB SPE and cellulose powder.
- Quantitative analysis was performed by analyzing all five drugs simultaneously from one plasma sample. The precursor ions and fragment ions for the five test compounds were as follows: alprazolam (m/z 309 → 281), atenolol (m/z 267 → 225), carbamazepine (m/z 237 → 194), diazepam (m/z 285 → 257), and sulfamethazine (m/z 279 → 186).

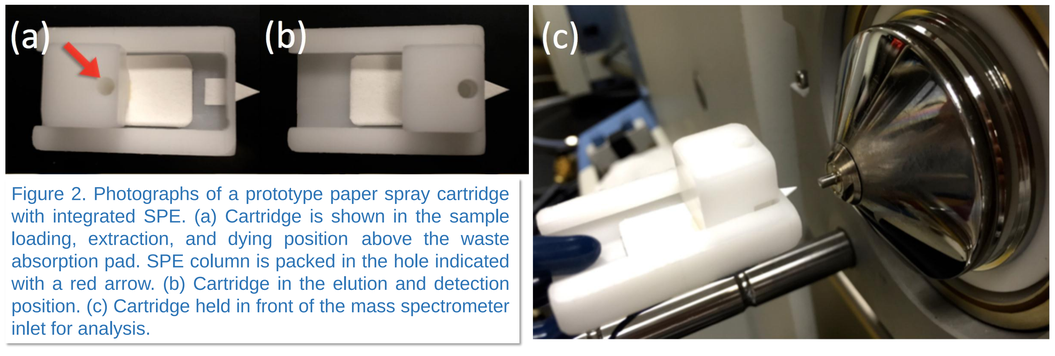


Figure 2. Photographs of a prototype paper spray cartridge with integrated SPE. (a) Cartridge is shown in the sample loading, extraction, and dying position above the waste absorption pad. SPE column is packed in the hole indicated with a red arrow. (b) Cartridge in the elution and detection position. (c) Cartridge held in front of the mass spectrometer inlet for analysis.

Results

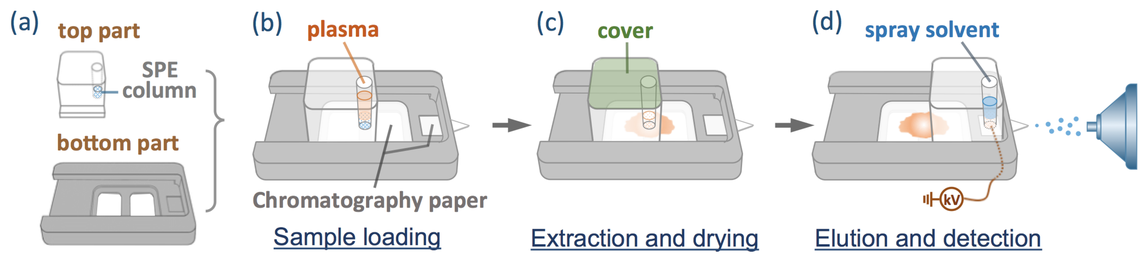


Figure 3. Diagram depicting the workflow for paper spray analysis with integrated on-cartridge solid phase extraction.

cartridge

The bottom part has two separate recessed regions to hold an absorbent waste pad and the paper spray substrate.

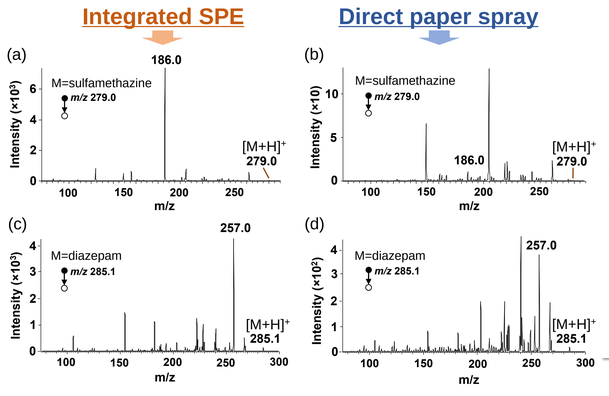
The top part has a hole bored through it to contain the SPE column.

Procedure

Sample loading: 10 µL up to hundreds of microliters plasma samples were added to the hole in the top part. The sample wicked through the SPE column and subsequently onto the absorbent pad.

Extraction and drying: the target analytes were retained on the SPE column while the excess matrix was absorbed onto the waste pad. A cover was used to prevent "backflow" during evaporation.

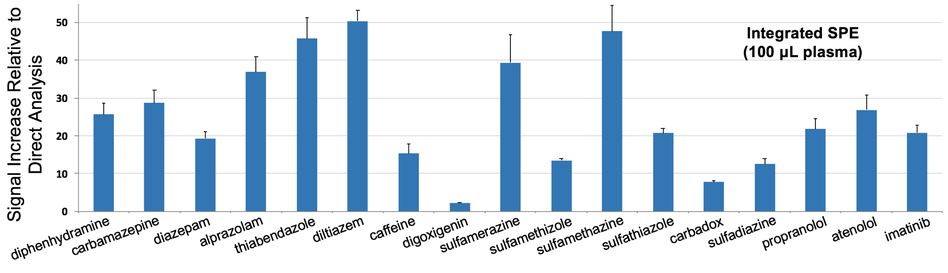
Elution and detection: The cartridge was positioned in front of the inlet to the mass spectrometer, and 60 µL extraction/spray solvent wicked through the SPE column, recovering the analytes in the process, and onto the spray substrate. Ionization occurs by inducing an electrospray at the sharp tip of the paper near the MS inlet.



Integrated SPE:

- Higher signal to noise (S/N)
- Higher absolute signal intensities

Figure 4. (a and c): Tandem mass spectra obtained for 200 ng/mL sulfamethazine and diazepam analyzed in 100 µL of plasma using paper spray with integrated SPE. (b and d): MS/MS spectra obtained when analyzing the same plasma sample using the typical paper spray approach directly on 3µL of sample.



Most drugs improved >20x

Figure 5. Relative increase in MS/MS signal intensity of the most intense fragment ion for 17 different drugs in plasma (200 ng/mL) analyzed by passing the 100 µL plasma sample through 5 mg of SPE material. Signal increase is relative to direct analysis of 3 µL of plasma by direct paper spray-MS/MS. Error bars are the standard deviation of the mean (N=5).

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y cartridge with integrated solid phase extraction for bioanalysis

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limits of detection

By using the SPE cartridge, the LODs of the five drugs each improved significantly, decreasing by a factor of 14 to 70, depending on the compound.

Detection limits could likely be further improved:

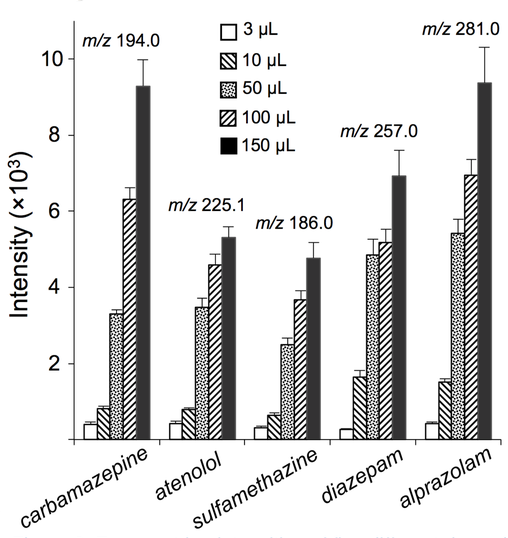
- increase the sample volume
- increase the amount of SPE material
- perform washing steps
- optimize the spray solvent

Table 1. Limits of Detection for Five Drugs by paper spray with integrated SPE compared with direct paper spray analysis

drug	integrated SPE LOD (ng/mL)	Direct analysis LOD (ng/mL)	factor decrease
carbamazepine	0.34	7.9	23
atenolol	2.2	58	26
sulfamethazine	0.08	5.2	70
diazepam	6.1	121	20
alprazolam	1.3	18.5	14

*100 µL sample and 5 mg SPE material were used.

Sample volume



Direct paper spray: increasing the volume of the sample significantly in order to improve the detection limits is not feasible.

Integrated SPE: larger sample volumes can be applied, signal intensity for each drug increased as the sample volume was increased.

Table 2. Signal Increase of Paper Spray with Integrated SPE Relative to Direct Paper Spray Analysis of a 3 μL Plasma Sample

sample volume (μL)	carbamazepine	atenolol	sulfamethazine	diazepam	alprazolam
3	3.3 ± 1.2[a]	2.8 ± 1.0	6.6 ± 2.5	1.9 ± 0.5	4.4 ± 1.1
10	6.8 ± 2.4	5.2 ± 1.8	13 ± 5.2	12 ± 4.8	16 ± 5.5
50	27 ± 4.5	23 ± 4.6	52 ± 10	36 ± 8.4	56 ± 11
100	52 ± 5.9	30 ± 4.2	77 ± 11	39 ± 5.5	72 ± 9.5
150	77 ± 15	35 ± 5.3	100 ± 21	51 ± 13	97 ± 23

[a]average value ± standard error of the mean (N=9)

Figure 6. Fragment ion intensities of five different drugs (200 ng/mL) in plasma obtained by on-cartridge SPE (5mg) paper spray ionization with plasma volumes of 3, 10, 50, 100, and 150 µL. Error bars show the standard deviation of the mean (N=8 at each volume)

Recovery and Ionization Suppression

paper spray with integrated SPE had **decreased ionization suppression** and **higher recovery** compared to direct paper spray.

3 µL Sample Volume		Alprazolam	Atenolol	Carbamaze.	Diazepam	Sulfametha.
Direct PS	Ion suppression	-72%	-50%	-66%	-68%	-69%
	Recovery	28%	30%	19%	30%	27%
PS on SPE cartridge	Ion suppression	-49%	-23%	-42%	-48%	-50%
	Recovery	70%	62%	29%	60%	57%
100 µL Sample Volume						
Direct PS	Ion suppression	-88%	-76%	-83%	-80%	-92%
	Recovery	18%	16%	22%	16%	24%
PS on SPE cartridge	Ion suppression	-57%	-27%	-52%	-55%	-67%
	Recovery	33%	65%	81%	29%	98%

Table 3. Ionization suppression and recovery for 5 different drugs in plasma. Analysis was performed with either direct paper spray analysis or using paper spray with integrated SPE. Three replicates were performed for each.

Quantitative analysis

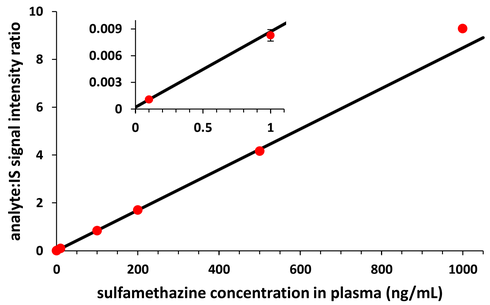


Figure 7. Calibration curve for sulfamethazine from 100 µL plasma sample using paper spray with integrated SPE (5 mg) using an isotopically labeled internal standard. Inset shows the two lowest calibration standards (0.1 and 1 ng/mL). Error bars show the standard deviation of replicate measurements of a single standard (N=3).

Quantitative performance:

- Good linearity from the LOD to 1000 ng/mL for each drug. (except atenolol, to 500 ng/mL)
- R2 of >0.99 was obtained in call cases.
- For each drug, the imprecision of the measurement less than 10% at all standard concentrations above the estimated lower limit of quantitation (10*sB/m).

Table 4. Regression parameters, lower limit of quantitation, bias, and imprecision for analysis of calibration standards prepared in plasma

	Atenolol		Alprazolam		Carbamazepine		Diazepam		Sulfamethazine	
Slope (m)	0.023		0.0075		0.013		0.010		0.0086	
intercept	0.0015		0.0022		0.000042		0.019		0.00021	
LOD (ng/mL)	2.2		1.3		0.3		6.1		0.08	
LLOQ[a] (ng/mL)	7		4		1		20		.2	
Standard (ng/mL)	% Bias[b]	%CV[c]	% Bias	%CV	% Bias	%CV	% Bias	%CV	% Bias	%CV
0.1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	0.6%	7%
1	< LOD	< LOD	< LOD	< LOD	3%	17%	< LOD	< LOD	-6%	8%
10	1%	4%	2%	9%	-13%	4%	1%	8%	6%	6%
100	-3%	1%	-2%	3%	-14%	2%	-2%	7%	-3%	7%
200	-8%	5%	-1%	5%	-11%	4%	-9%	2%	-2%	3%
500	10%	8%	1%	2%	-6%	5%	-1%	7%	-3%	2%
1000	>ULOQ	>ULOQ	8%	5%	14%	2%	11%	3%	8%	0.1%

[a]estimated LLOQ. LLOQ = $10*s_b/m$
[b]% bias = (calculated concentration – actual concentration)/actual concentration*100%
[c]% CV = relative standard deviation of replicate measurements of a single standard (N=3)

Conclusions

- The aim of this work was to create an “all-in-one” disposable capable so that sample extraction, pre-concentration, and ionization could be performed from a single device.
- Compared to direct paper spray, the integrated SPE approach:
  - improved the MS signal intensity and detection limits significantly.
  - have lower levels of ionization suppression and better overall recovery.
- The five drugs tested showed good linearity when an isotopically labeled internal standard was spiked into the sample.
- The simple and automatic on-cartridge preparation could also enable more effective use of portable mass spectrometers or the use of mass spectrometers outside traditional analytical labs.

Acknowledgements

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