ASMS 2004

HIGH THROUGHPUT SCREENING OF SMALL MOLECULAR WEIGHT NEW CHEMICAL ENTITIES AND NON-COVALENT BINDING OF SMALL MOLECULES TO INTACT PROTEINS USING MALDI-TOF AND DIOS TARGET PLATES

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OVERVIEW

Desorption/ionization on silicon (DIOS)[1] method uses the porous silicon surface to trap the analyte molecules. Because of its high absorption in the ultraviolet, the surface acts as an energy receptacle for the laser radiation.

The ability to perform analysis without a matrix makes it more amenable to small molecule analysis.

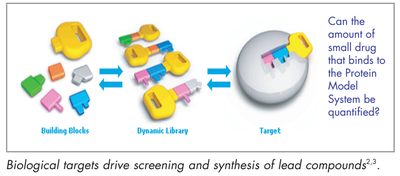
In this study, DIOS is used for screening and quantifying of small molecular weight new chemical entities (NCEs) and the non-covalent binding of small molecules to a protein model system.

INTRODUCTION

Pharmaceutical and biotechnology companies are racing to identify and test NCEs that may be turned into therapeutic drugs.

As the number of drug targets and NCEs is increasing dramatically, there is a need for quick, efficient and high throughput screening (HTS).

In this study, we investigated if DIOS-TOFMS is suitable for NCE HTS.



Biological targets drive screening and synthesis of lead compounds[2,3].

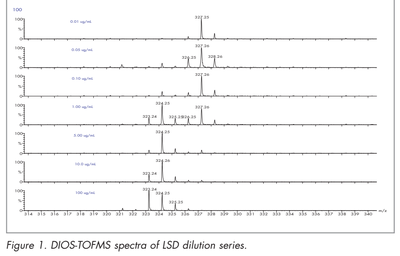


Figure 1. DIOS-TOFMS spectra of LSD dilution series.

METHODS

DIOS Plates and Sample Preparations

DIOS Plates:	Waters MassPREP™ DIOS-target treated with HPLC grade IPA overnight prior to use
Sovlents:	50/50 ACN/H2O was used for all small molecule quantitation experiments and 20 mM ammonium acetate buffer solution for ligand/protein binding experiments
Samples:	For all experiments, 0.5 µL of sample was applied to DIOS plate wells and dried under ambient conditions
Denatured Proteins:	Proteins were denatured on DIOS plate by addition of organic and/or acidic solvents on top of the samples
Internal Standards:	Deteurated compounds were used as internal standards for all small molecule quantitation experiments, reserpine (5 µM/mL) was used as internal standard for ligand/protein binding experiments

DIOS-TOFMS

Instrument:	Waters M@LDI-LR®
Source Voltage:	15 KV
Delay time:	500 ns
Laser:	337 nm Nitrogen Laser from LSI (Franklin, MA)
Laser Rate:	10 Hz
Mode:	Positive ions in Reflectron mode
Detector:	MCP

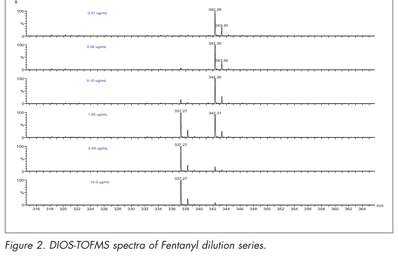


Figure 2. DIOS-TOFMS spectra of Fentanyl dilution series.

RESULTS

DIOS-TOFMS spectra for the Lysergic Acid Diethylamide (LSD) and Fentanyl dilution series experiments with the concentration range from 0.01 µg/mL to 100 µg/mL are reported in Figures 1 and 2. Peak 324 (m/z) is LSD and peak 327 (m/z) is LSD-d3, the internal standard at 1.00 µg/mL. Peak 237 (m/z) is Fentanyl and peak 242 (m/z) is Fentanyl-d5, the internal standard at 1.00 µg/mL. All data was acquired, processed and quantified automatically using Masslynx and its application managers and a customized software program.

Table 1 and table 2 summarized the LSD and Fentanyl calibration curves from 0.01 µg/mL to 10 µg/mL. Each data point was run in quadruplet for LSD and duplicate for Fentanyl. The peak intensity ratios of 324/327 for LSD and 237/242 for Fentanyl were used as the signal (peak area can also be used with almost identical results). It appeared that the linear fit for the calibration curve can only be obtained from 0.01 µg/mL to 10 µg/mL. The LSD and Fentanyl calibration curves are reported in Figures 3 and 4 respectively. Very good linearity was obtained from both Curves with R^2=0.9999 and R^2=0.9997. The detection and quantitation limit for both small drugs were 5 pg on DIOS plate.

	DIOS-TOFMS for LSD				
Concentration	Peak Ratio 1	Peak Ratio 2	Peak Ratio 3	Peak Ratio 4	Peak Ratio Average
0.01	0.04	0.03	0.04	0.04	0.04
0.05	0.21	0.14	0.18	0.14	0.17
0.10	0.22	0.18	0.21	0.18	0.20
0.50	0.74	0.69	0.67	0.77	0.72
1.00	1.26	1.29	1.27	1.30	1.28
5.00	6.26	6.60	6.42	6.75	6.51
10.00	12.28	12.92	12.08	12.35	12.41

Table 1 DIOS-TOFMS LSD dilution series quadruplet experiment results.

	DIOS-TOFMS for Fentanyl		
Concentration	Peak Ratio 1	Peak Ratio 2	Peak Ratio Average
0.01	0.01	0.03	0.02
0.05	0.15	0.08	0.12
0.10	0.15	0.15	0.15
0.50	0.75	0.69	0.72
1.00	1.10	1.10	1.10
5.00	5.28	5.66	5.47
10.00	10.99	11.4	11.20

Table 2. DIOS-TOFMS Fentanyl dilution series replicates experiment results.

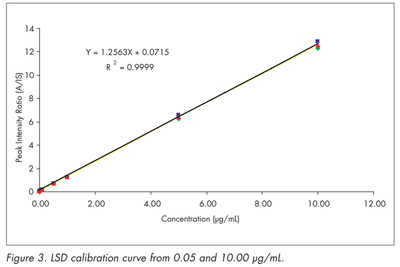


Figure 3. LSD calibration curve from 0.05 and 10.00 µg/mL.

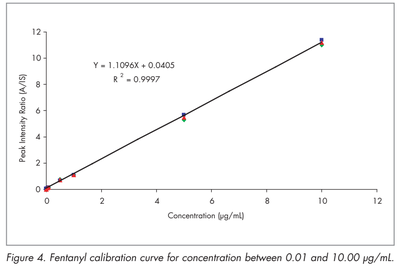


Figure 4. Fentanyl calibration curve for concentration between 0.01 and 10.00 µg/mL.

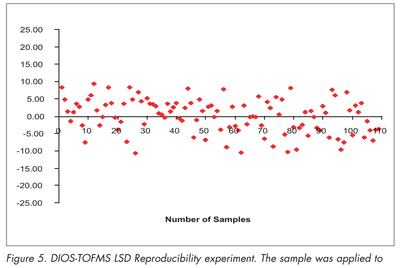


Figure 5. DIOS-TOFMS LSD Reproducibility experiment. The sample was applied to 96 wells DIOS plate. 110 data points were collected from those wells. The calculated standard deviation of the experiment was about ±10%.

Other standards drugs (Desipramine, Diphenhydroamine, Chlorpheniramine, Erythomycin and Verapamil) were also used to generate standard calibration curves (deteurated analogs used as internal standards) using the DIOS-TOFMS system. Similar results were obtained from those standard small drugs.

In summary, the DIOS-TOFMS can be used for small molecules quantitation when deteurated analogs were used as internal standards. Using the DIOS-TOFMS system, more than 200 NECs have been screened. If the spotting process were automated with a Waters MassPREP station, for example, total analysis time could be less than a minute per sample.

A DIOS-TOFMS spectrum for Warfarin [Coumafene 4-Hydroxy-3-(3-oxo-1 phenylbutyl) coumarin, $C_{19}H_{16}O_4$] is reported in Figure 6. The molecular weight of Warfarin is 308.33. Warfarin was detected as the sodium adduct $[M+Na]^+$. The Warfarin calibration curve with the concentration between 0.05 µM/mL to 10.00 µM/mL with good linearity (R^2=0.9984) is reported in Figure 7. Reserpine was used as the internal standard for the experiment. A pair of comparison spectra of Warfarin before and after the protein model was denatured is reported in Figure 8. The calibrated value from the calibration curve for Warfarin was 0.444 µM/mL, which is about 11.11% off from the real concentration of 0.500 µM/mL Warfarin in the sample.

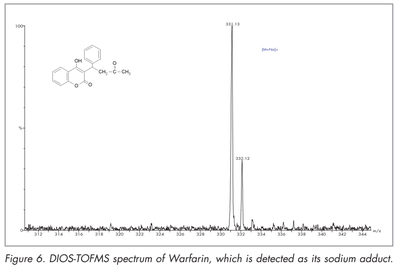


Figure 6. DIOS-TOFMS spectrum of Warfarin, which is detected as its sodium adduct.

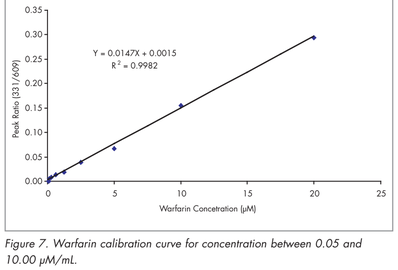


Figure 7. Warfarin calibration curve for concentration between 0.05 and 10.00 µM/mL.

CONCLUSION

DIOS-TOFMS can be used for high throughput screening of small molecule weight new chemical entities (NCEs) with the possibility of elemental composition analysis for the by-products of NCEs.

The detection and quantitation limits for those small molecule drugs (such as LSD, Fentanyl, etc.) were about 5 pg on DIOS target plate. The linearity with deteurated analogs as internal standards for such small molecule drugs was 3 orders of magnitude.

DIOS-TOFMS can be used for non-covalent binding of small molecules to intact proteins studies by denaturing the proteins on the DIOS target plate to release the small molecules. Non-covalent binding constant for model protein system could be obtained by quantifying the small molecules.

ACKNOWLEDGEMENT

Waters Corporation:	Dr. Gordon Fujimoto
	Dr. Jeanne Li
Atlantos, Inc.:	Dr. Irving Sucholeiki

REFERENCES

1. Wei, J. , Buriak, J. & Siuzdak, G. (1999) Nature (London) 399, 243-246.
2. Lehn, J. M. & Eliseev, A. V. (2001) Science 291, 2331-2332.
3. Ramström, O. & Lehn, J. M. (2002) Nature Reviews, Drug Discovery 1, 26-36.

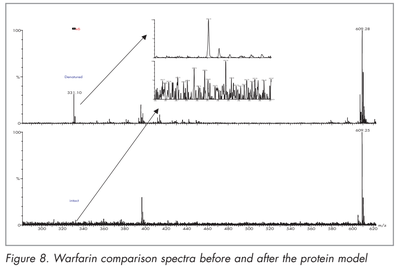


Figure 8. Warfarin comparison spectra before and after the protein model system was denatured. No proteins present.

May 2004 720000882EN LLPDF