Strategy for predicting molecular coverage and enhancing successful analysis for CACO2 studies in high-throughput LDTD-MS/MS

WP626

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SCOPE

Mass spectrometry users are familiar with electrospray ionization properties. They develop knowledge on the behavior of the technique upon different molecular structures and strategies to obtain successful ionization. Usage of the Laser Diode Thermal Desorption ion source is increasing throughout the field as it reduces the analysis time to a few seconds per sample. Fundamental physics phenomenons of thermal desorption are different from electro-nebulization process. The scope of this study is to identify molecular functional groups which play a role in thermal desorption and to investigate the possibility of improving vaporization efficiency.

INTRODUCTION

Drug discovery laboratories test high numbers of molecules which contain various structures. Quantitation of such drugs requires high-throughput mass spectrometry techniques. UPLC system is currently the gold standard for these analyses and compounds are separated on properties such as acid/base and polarity to achieve adequate separation and ionization. Increasing throughput remains of high interest in this field. Use of Laser Diode Thermal Desorption ion source reduces the analysis time to 8 seconds per sample compared to minutes by UPLC-MS/MS. Methodology for prediction of vaporization and ionization success relies on different properties than those of liquid chromatography.

LDTD™ Ionization Source:

The LDTD uses a Laser Diode to produce and control heat on the sample support **(Figure 1)** which is a 96 wells plate**.** The energy is then transferred through the sample holder to the dry sample which vaporizes prior to being carried by a gas in a corona discharge region. High efficiency protonation and strong resistance to ionic suppression characterize this type of ionization, and is the result of the absence of solvent and mobile phase. This allows for very high throughput capabilities of 8 seconds sample-to-sample analysis time, without carry over.

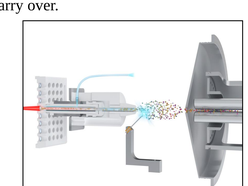

Figure 1 Schematic of the LDTD ionization source

METHOD

A panel of 35 compounds with various structural characteristics is analyzed with LDTD-MS/MS. 2 µL of pure solution at 2.5 µM is deposited in 96-wells plate and dried. A full scan spectrum is acquired in positive and negative mode. Product ion scan of the most intense molecular ion is then made and MS/MS transition is chosen for subsequent tests. Structural characteristics of molecules are analyzed with Pallas™ software for the prediction of the local pKa of the functional group. Upon observations, application of additive substances on desorption plate is tested to validate the predicted vaporization behavior. The compounds are pooled in a CACO2 buffer solution and diluted 10x prior analysis. Quantitation is achieved for the different plate treatments.

RESULTS

Background:

- Thermal Desorption behavior is governed by the effective enthalpy of volatilization characteristics of a molecule.
- LDTD ion source use nano-scale physical properties and gas dynamics to reduce the vaporization energy.
- Strong polarity functional group still acts on the ability of a compound to vaporize in LDTD process.

Carboxylic Acid:

- In a pure solution, we observe a quadratic response with respect to concentration (**Figure 2**).
- Hypothesis: Formation of salt while the solution dries with remaining counter ion.
- Adding EDTA into the solution or in plate pre-coating prevents the binding of the compound and allows normal linearity, sensitivity and reproducibility. This has been used since 2010.
- Example of hydroxy-diclofenac shows 10x improvement in signal and linear behavior in presence of EDTA

Example: Hydroxy-Diclofenac

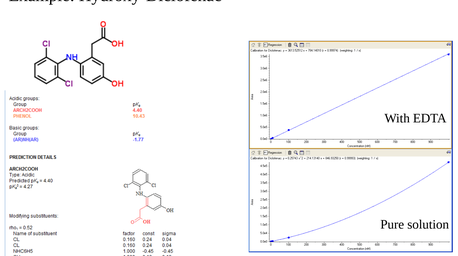

Figure 2 LDTD analysis of OH-Diclofenac from 1 to 1000 nM

Phenol group:

- Despite high local pKa value calculated, vaporization shows a good performance.
- Analysis of Dextrorphan in pure solution is normal even with a phenol group showing local pKa of 9.48 (**Figure 3**)

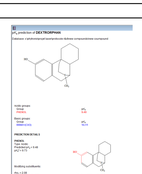

Figure 3 Dextrorphan pKa prediction

Other Acidic group:

- A functional group of type RRCHOH with a high pKa alone is generally not sufficient to disturb desorption efficiency.
- A presence of 2 groups or more presents the same behavior as carboxylic acid at a higher extent
- Adding EDTA into solution or in plate pre-coating prevents binding of the compound and allows normal linearity, sensitivity and reproducibility.
- Example: Metoprolol RRCHOH pKa (**Figure 4**) alone desorbed well with LDTD. Doxycycline absolutely needs EDTA to vaporize with its multiple acid functions.
- Similar structure of benzodiazepines clearly shows the consequence of two acid functions (**Figure 5**) as only Oxazepam needs EDTA for analysis.

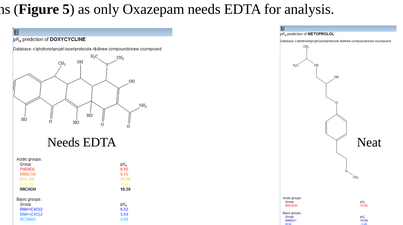

Figure 4 Doxycycline and Metoprolol pKa prediction

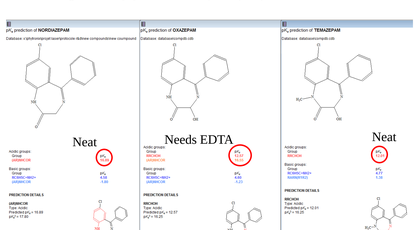

Figure 5 Nordiazepam, Oxazepam and Temazepam pKa prediction

Glucuronide ring:

- Multiple high local pKa values ranging between 15-19 corroborate the absence of vaporization for glucuronide compounds.
- Adding EDTA is not sufficient to obtain efficient desorption conditions.

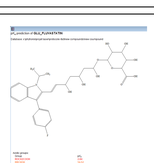

Figure 6 Fluvastatin glucuronide pKa prediction

Basic group:

- A basic functional group with a high pKa alone is generally not sufficient to disturb desorption efficiency.
- Observed effect is less frequent than for an acidic function.
- Example: Clomiphene RRRNH+B pKa (**Figure 7**) alone desorbed well with LDTD. Spermidine will only vaporize in presence of TETA (Triethyltetramine).
- Chloroquine desorbs efficiently even with 2 basic groups.

Figure 7 Clomiphene, Spermidine and Chloroquine pKa prediction

Other observed particular functions:

Tertiary amine on cycle:

- Compound with tertiary amine on cycle express quadratic behavior in pure solution.
- Trace residue of protein precipitation from BSA allows adequate LDTD analysis.
- Same behavior for erythromycin and clarithromycin, which have the same functional group.

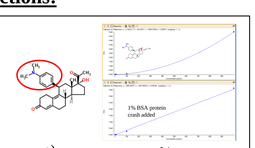

Figure 8 a)Ulipristal structure and b) LDTD analysis of Mifepristone

Amino Acid:

- Acidic and basic function combined limit the vaporization efficiency.
- EDTA combined with TRIS buffer allowed first amino acid quantitation with LDTD analysis.
- Still under development for improvement in sensitivity.

Ribose sugar:

- Adenosine contains ribose sugar molecule with 3 high value pKa.
- Quantitation is obtained by adding HEPES buffer, a zwitterionic organic chemical buffering agent.
- Mechanism of action under investigation

Discussion and Conclusion

- **Small molecule coverage working without any additive shows 85% success rate (ASMS 2010 ThOF).**
- **Systematic addition of EDTA, as there is no adverse effect, rose the coverage at 95%**
- **Several functional groups influencing desorption efficiency have been identified.**
- **Additive substances enhancing the thermal desorption were found.**
- **Other improvements on mechanism knowledge based on molecular structure are foreseeable in the future.**