Generic Methods for Diuretic Determination in Urine using Log D and pH Prediction Liquid/Liquid or Solid Phase Extraction with SPE Cartridges and HPLC Analysis

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Introduction

In the last few years diuretics have been misused and abused in sports where categories are involved, to reduce weight prior to a competition or to deliberately dilute the urine specimen as a tentative attempt to escape the drug test. Also, these compounds are prohibited by the International Olympic Committee.

These molecules are polar (acidic and basic in nature) and are relatively difficult to get good limit of detection (LOD) from biological fluid by using HPLC analysis. In order to improve LOD of targeted diuretics, the extraction methods need to be precisely determined based on the molecular structure of each one. This paper presents some pathways for method extraction of Furosemide and Ethacrynic Acid by SPE with SiliaPrep™ cartridges or liquid/liquid extraction for urine matrix according to Log D versus pH curve generated by the Pallas Combi™ software.

Furosemide and Ethacrynic Acid

Pallas Combi™ Software

The Pallas Combi™ is a prediction software to help analytical chemists to:

- Acquire a better understanding of chemical properties
- Facilitate extraction
- Help in the selection of a suitable internal standard
- Overall reduction of R&D method development time
- Methods (extraction-analysis) are more robust

Pallas uses the Log D, which is the log distribution coefficient (solubility from octanol/water ratio) at a particular pH. This value is not constant and will vary according to the protogenic nature of the molecule.

The more positive is a Log D value for a compound, the more it is soluble in non-polar or organic solvent and the more it is retained on a reversed phase column.

The more negative is a Log D for a compound, the more it is soluble in polar or watery solvent and it is less retained on a reversed phase column.

Method Extraction Results (% Recovery by HPLC)

<table>
<thead>
<tr>
<th>DIURETIC</th>
<th>LIQUID/LIQUID*</th>
<th>SPE SiliaPrep C18 (500mg)***</th>
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<tbody>
<tr>
<td>Furosemide</td>
<td>58.1%</td>
<td>71.3%**</td>
</tr>
<tr>
<td>Ethacrynic Acid</td>
<td>105.5%</td>
<td>101.2%***</td>
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</tbody>
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Log D Parameter

Log D higher than 2:

More soluble in organic solvent. Solid Phase Extraction (SPE) not recommended, only liquid/liquid extraction.

Log D between -2 and 2:

Good affinity with aqueous solvent — Ideal for reversed phase chromatography (HPLC). Solid phase extraction (SPE) is recommended for extraction of the analyte.

Log D lower than -2:

Zone very hydrophilic. SPE ion-exchange is recommended and polar HPLC column is necessary (HILIC approach). No liquid/liquid extraction.

pH Parameter

The best pH given by Pallas is where the curve is typically flat. With a flat curve, the recovery from extraction is more reproducible and the robustness of the HPLC method is improved.

Pallas Curve for Furosemide

- SPE extraction at pH 5.0 and lower

Pallas Curve for Ethacrynic Acid

- Acidic extraction at low pH

Conclusion

This paper demonstrates the relationship between the curve from Pallas and the results obtained for the extraction of Furosemide and Ethacrynic Acid in urine matrix. According to the curve of Pallas for Furosemide, SPE extraction gives better recovery compared to the liquid/liquid extraction because the Log D is typically in the region for SPE extraction (slightly acidic). For Ethacrynic Acid, Pallas curve shows high value for Log D (more than 3) and the recovery from liquid/liquid and SPE were done in acidic condition to give total recoveries.

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