A Novel Separation for the Bath Salts using a Multi-Mode Reversed-Phase Column

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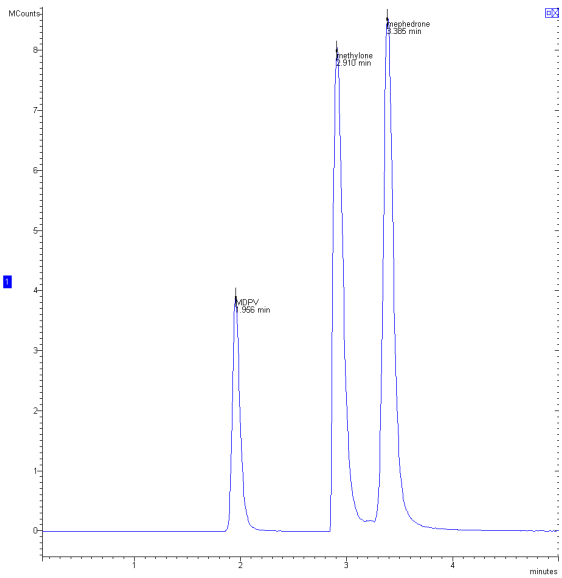
Analysis and separation of bath salts mephedrone, methylone and MDPV was achieved using a multi-mode reverse phase column. The separation was obtained using the column in aqueous normal phase (ANP) mode. Bath salts were separated in four minutes using an isocratic mobile phase composed of acetonitrile and ammonium formate. The separation was effected by the concentration of acetonitrile, the buffer concentration and the pH of the aqueous buffer. Increasing the organic modifier concentration caused an increase in retention time of the analytes. Increasing the pH caused the analyte retention time to decrease. Using these parameters, a rapid and simple method was developed that could quantitate the bath salts. When the analytes were separated on a reversed phase stationary phase the order of elution was methylone, mephedrone and MDPV. When the separation was performed using the multi-mode reverse phase stationary phase in the ANP mode, the order of elution was MDPV, methylone and mephedrone (Figure 1). This indicated that the main mechanism of separation was the charge on each molecule, than the hydrophobicity. Urine samples were analyzed using this method after solid phase extraction clean up. It is important to note that the samples were reconstituted in acetonitrile prior to LC/MS analysis. This had two advantages. 1) The high acetonitrile concentration prevented peak broadening under the isocratic conditions. 2) Use of acetonitrile allowed for better solvation of ions, which increased the sensitivity of the assay. The high organic modifier concentration prevented retention of various non-polar compounds that would lead to matric interference. Column equilibration is important. It is important to ensure that the stationary phase is at the proper pH. Irreproducible results are obtained if the column is not at the proper pH prior to the analysis.



Chromatogram of MDPV, methylone and mephedrone separated using a multi-mode reversed phase column with a 90:10 acetonitrile/ammonium acetate pH 6.8 isocratic mobile phase.