Method Development for Precise Quantification of Amino Acids in Sports Drinks Using HPLC

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Purpose:
A single method that could simultaneously quantify multiple amino acids in simple solutions (such as sports drinks) would prove useful in extending physiological and dietary research of the amino acids used in these formulations. This method requires less derivatization than some current methods used to analyze physiological samples.

TLC PROCEDURE

Samples are spotted at the bottom of the TLC plate.

The TLC plate is placed over the developing chamber and the solvent travels up the plate along with the sample.

The TLC plate is then removed with developing chamber.

TLC plate has been developed and scanned for developing chamber.

FUTURE RESEARCH:

Future research will involve testing more amino acids on HPLC.

With 1/10 diluted DNFB produced:

The 1/10 diluted (in phosphate buffer) produced:

Unreacted DNFB.

Taurine standards

The 5.2:8.0:2.0:2.0 V:V acid:water solvent system

Taurine standards

The Rf acid:water

Consumers in 2017.

The Amino Acid Database.

The Optimization of Amino Acid Distribution in L-Lysine by Amino Acid Composition of Microorganism.

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Figure 1: The solvent systems used, from left to right, were 96:24:40 V:V:n-propanol:ethanol:water, 96:24:40 V:V:n-propanol:ethanol:water:acetic acid, and 5.2:8.0:2.0:2.0 V:V acid:water:acetic acid solvent system.

Figure 2: Chromatogram of a taurine standard run at room temperature.

The first major peak corresponds to 2,4-dinitrophenol, the second peak corresponds to the DNP taurine derivative and the last major peak corresponds to unreacted DNFB. The first minor peak corresponds to a likely byproduct of the derivatization process.

Figure 3: Chromatogram of the 1/10 diluted 10 ppm taurine standard. The first major peak corresponds to 2,4-dinitrophenol, the second peak corresponds to the DNP taurine derivative and the last major peak corresponds to unreacted DNFB. The first minor peak corresponds to a likely byproduct of the derivatization process.

Figure 4: Chromatogram of a single amino acid standard. The first peak is a 2,4-dinitrophenol, the second peak is the DNP amino acid derivative and the last peak is unreacted DNFB. The first minor peak corresponds to a likely byproduct of the derivatization process.

Figure 5: Both the absorption of dilute (1/10 20 ppm taurine standard diluted in phosphate buffer) and undiluted 20 ppm taurine standards are run using UV-Vis spectrophotometry. The undiluted 20 ppm taurine sample mixed out at the limit of the UV detector. The dilute taurine standard remained well below the maximum absorption value and peaks at 360 nm, the expected maximum absorption for a 2,4-dinitrophenol amino acid derivative.

Figure 6: Linear relation of concentration and peak area of the Taurine standard. The R^2 value is .9990

Figure 7: Linear relation of concentration and peak area of the diluted 1/10 20 ppm Taurine standard. The R^2 value is .9990

Figure 8: Linear relation of concentration and peak area of the undiluted 20 ppm Taurine standard. The R^2 value is .9990

Figure 9: Linear relation of concentration and peak area of the diluted 1/10 20 ppm Taurine standard. The R^2 value is .9990

Figure 10: Linear relation of concentration and peak area of the diluted 1/10 20 ppm Taurine standard. The R^2 value is .9990

Future Research:

• Testing more amino acids on HPLC
• Test simultaneous amino acids on HPLC
• Identify amino acids in TLC based on color and Rf

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CONCLUSIONS:

TLC Conclusions:

• Valid method of separation and no overlapping Rf values
• The 5.2:8.0:2.0:2.0 V:V:n-propanol:ethanol:water:acetic acid solvent system was best in comparison to the other solvent systems

HPLC Conclusions:

• Initial results for HPLC testing yielded unusable data
• Color intensity produced by the DNP derived taurine was too high
• The 1/10 diluted (in phosphate buffer) produced linearly correlated peaks and the R^2 value was 0.9990
• Taurine standards derivatized with 1/10 diluted DNFB produced linearly correlated peaks. The R^2 value was 0.9964
• The R^2 values produced by these trials indicates the potential of a robust and precise method

Citations: