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# Development and Application of LC/HRPS for Quantification of Adenine Nucleotides, Creatine Phosphate, and Creatine in Sturgeon Spermatozoa

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## Supplementary Online Material (SOM)

Table S1. Liquid chromatography gradient for the separation of target analytes

| Time (min) | A | B | Flow (µl/min) |
|---|---|---|---|
| | (%) | | |
| 0.00 | 98.0 | 2.0 | 200 |
| 2.00 | 98.0 | 2.0 | 200 |
| 4.00 | 85.0 | 15.0 | 300 |
| 8.00 | 50.0 | 50.0 | 400 |
| 10.00 | 50.0 | 50.0 | 400 |
| 10.01 | 98.0 | 2.0 | 250 |
| 13.00 | 98.0 | 2.0 | 250 |

A = water + 2 mmol/l ammonium acetate + ammonia, B = acetonitrile + 2 mmol/l ammonium acetate + ammonia

Table S2. Mass spectrometry/high resolution mass spectrometry parameters

| Analyte | Mode | Parent ion | Quantification transition | Confirmation transition | NCE (%) | RT (min) |
|---|---|---|---|---|---|---|
| | | (*m/z*) | | | | |
| CP | – | 210 | 78.9591 | 96.9696 | 35 | 1.11 |
| Creatine | – | 130 | 88.0404 | * | 35 | 3.76 |
| AMP | – | 346 | 78.9591 | 134.0472 | 30 | 6.09 |
| ADP | – | 426 | 134.0472 | 328.0453 | 30 | 6.31 |
| ATP | – | 506 | 158.9253 | 408.0124 | 30 | 6.35 |
| cAMP | + | 330 | 136.0618 | 312.0489 | 30 | 7.91 |

CP = creatine phosphate, AMP = adenosine monophosphate, ADP = adenosine diphosphate, ATP = adenosine triphosphate, cAMP = cyclic adenosine monophosphate, RT = retention time, NCE = normalized collision energy

*no confirmation ion