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研究獎學金 Research Scholarship



以液相層析串聯式質譜技術檢測蜂王乳中的單、雙、三磷酸腺苷化合物 Quantification of Adenosine Mono-, Di- and Triphosphate from Royal Jelly using Liquid Chromatography - Tandem Mass Spectrometry



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研究重點

Nucleotides are composed of nitrogenous base, ribose and phosphate groups. Adenine (Ade), adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP) all play important roles in physiological metabolism. Royal jelly is the worker bee's secretions, it is rich in a variety of pure natural ingredients, several studies have shown that royal jelly can supply abundant nutrition to the human. In this study, a rapid and effective LC/MS method coupled with two type of pre-processing methods were developed and validated for the accurate quantification of Ade, AMP, ADP and ATP contents in royal jelly. To achieve the best extraction efficiency, two pretreatment methods, solid-phase extraction (SPE) and dispersive solid-phase extraction (dSPE), were developed and investigated. The silica-based cyanopropyl (CN) liquid chromatography was employed using pH programming with quaternary mobile phase system. The total LC/MS run time was within 12 min with a constant flow rate of 0.25 mL/min. The linear range were 2.5-1000 ng/mL with correlation coefficient $r = 0.9995$. The limit of detection (LOD) and limit of quantitation (LOQ) of 1.0 ng/mL and 2.5 ng/mL were achieved, respectively. Precision (RSD% < 10.5%) and accuracy (recovery 81.3-118.4%) were both satisfactory in two pre-processing methods. Eventually, nucleotides were successfully quantified from the 2-day and 3-day royal jelly samples, with concentrations within 6.2 to 2126.0 mg/kg.

研究成果

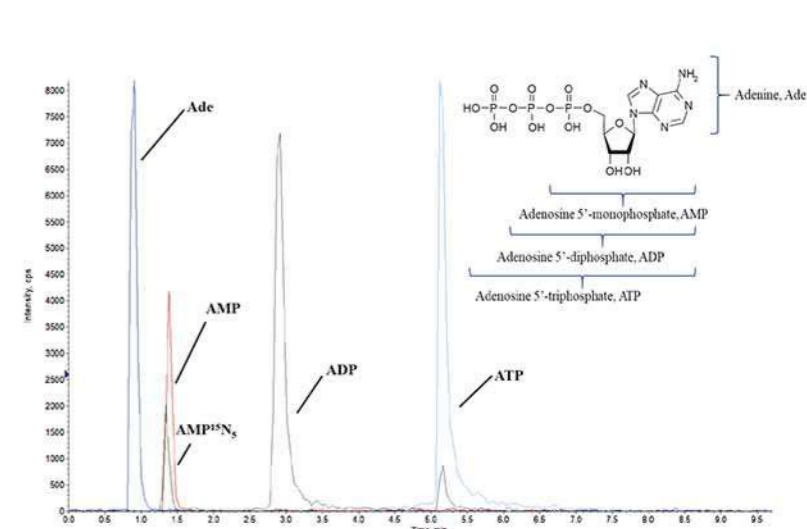
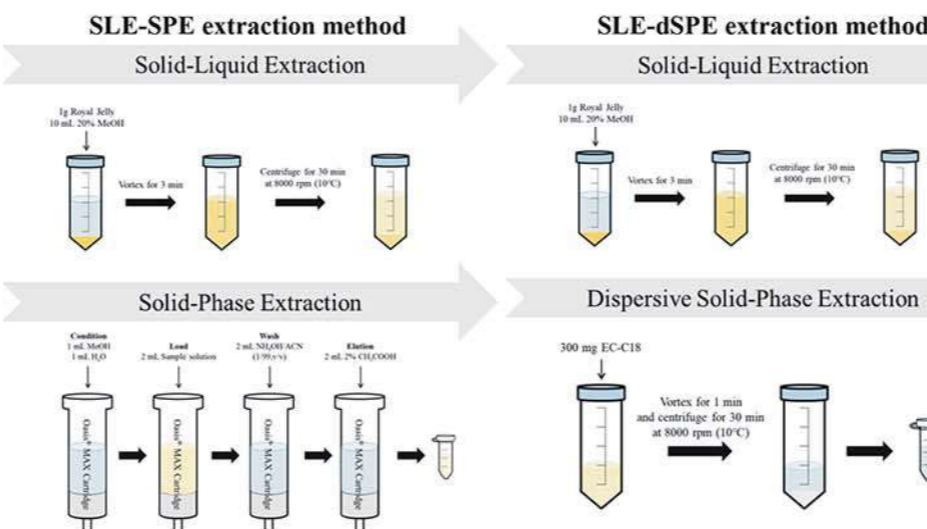
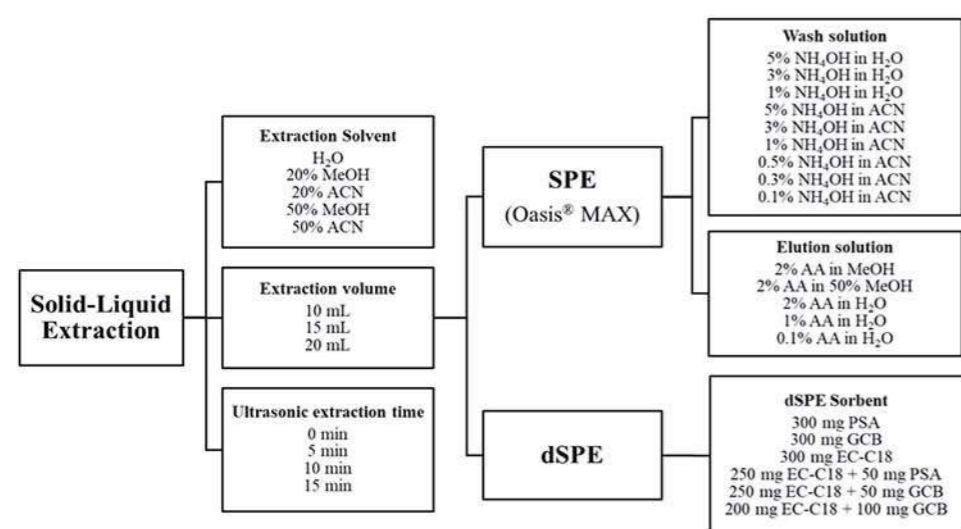


Figure 1. The overall workflow for optimization of SLE-SPE and SLE-dSPE extraction method strategy.

Figure 2. The overall experimental workflow for quantitative analysis. Figure 3. MRM spectra of mixed-standard solution.

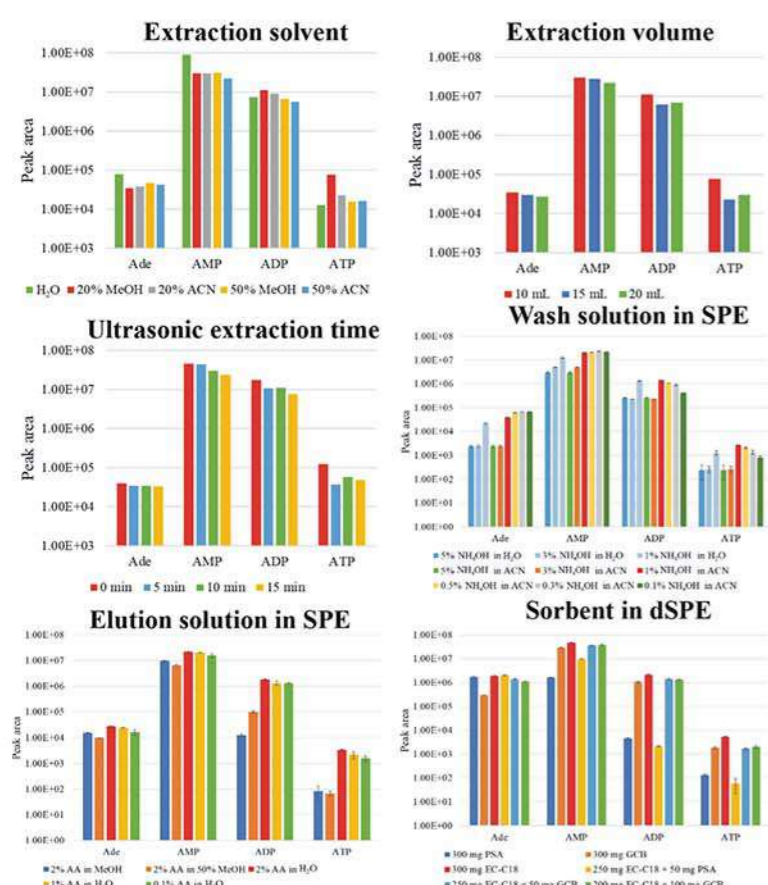


Figure 4. Comparisons of peak areas of analytes by optimizing pre-processing parameters using royal jelly.

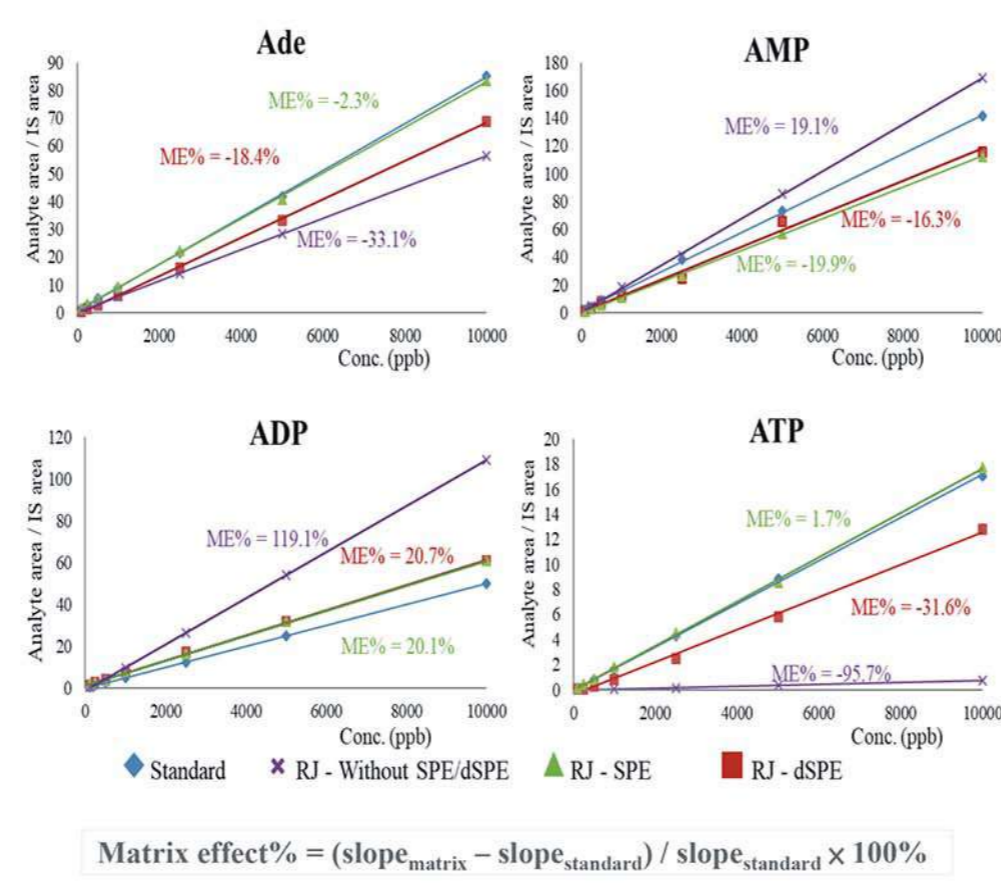


Figure 5. Matrix effects of royal jelly matrix solution with/without SPE or dSPE by the slopes of matrix-matched calibration curves.

Table 1. Linearity of the standard calibration curves. (n = 3)

Linear equation	Linear range (ppb)	Coefficient of determination (r)	LOD (ppb) ^a	LOQ (ppb) ^b
ATP Y = 0.00219 X - 0.0185	10 - 1000	0.9996	5	10
ADP Y = 0.00465 X - 0.0135	5 - 1000	0.9998	2.5	5
AMP Y = 0.015 X + 0.00867	2.5 - 1000	0.9999	1	2.5
Ade Y = 0.00156 X + 0.00407	2.5 - 1000	0.9995	1	2.5

^a S/N ≥ 3, ^b S/N ≥ 10

Table 2. Quantification of Ade, AMP, ADP and ATP from 2-day and 3-day royal jelly by dSPE (standard addition method)

	Linear equation	Coefficient of determination	Contents (mg/kg)
3-day royal jelly			
ATP	Y = 0.000435X + 0.0493	0.9990	11.3
ADP	Y = 0.00689X + 9.96	0.9985	144.6
AMP	Y = 0.00969X + 206	0.9967	2126.0
Ade	Y = 0.00620X + 0.0429	0.9994	6.9
2-day royal jelly			
ATP	Y = 0.0000735X + 0.00826	0.9975	11.2
ADP	Y = 0.00746X + 11.3	0.9985	151.5
AMP	Y = 0.01110X + 201	0.9952	1810.8
Ade	Y = 0.00488X + 0.033	0.9983	6.2

Liao, W. R., Huang, J. P., & Chen, S. F. (2020). Quantification of adenosine Mono-, Di- and triphosphate from royal jelly using liquid chromatography-Tandem mass spectrometry. *Journal of Food and Drug Analysis*, 28(3), 365.

研究生生活及心得

感謝財團法人中技社研究獎學金的肯定，給予我莫大的動力與信心。研究的路程中，總伴隨著挫折、失望感，但越過瓶頸後，所獲得的成就感，總能讓疲憊與辛勞在那瞬間消失殆盡，我想這就是做研究最吸引人的地方。碩博班期間，感謝陳頌方教授對我的指導，在我研究上遇到困惑時，總是會適時提供我協助，並讓我有機會接觸生醫相關研究，使我將所學應用到不同領域上。最後，期許自己能夠更加精進，將所學回饋社會。



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