Analytical Methods

HPLC determination of adenosine in royal jelly

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ABSTRACT

A simple method is described for the determination of adenosine in royal jelly. The adenosine in the sample was extracted using 80% ethanol and analysed by reversed-phase high-performance liquid chromatography (HPLC). Chromatographic separation was performed using a Dionex HPLC system with a Waters Symmetry C18 column and gradient elution with a mixture of two solvents: solvent A, 0.4% phosphoric acid and solvent B, methanol. The effluent was monitored using a UV detector set at 257 nm. The average recoveries were 91.6–98.3% ($n = 6$) with standard deviation below 5.3%. The limits of detection

adenosine from RJ using ultrasonic-assisted extraction. The different factors affecting the efficiency of the extraction such as extraction solvent and extraction time were carefully optimised.

Capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) are currently the most commonly used separation techniques for adenosine in combination with detection by ultraviolet (Gong et al., 2004; Kieszling et al., 2004; Tzeng, Hung, Wang, Chou, & Hung, 2006), diode array and evaporative light-scattering (Yan, Luo, Wang, & Cheng, 2006), and mass spectrometry (

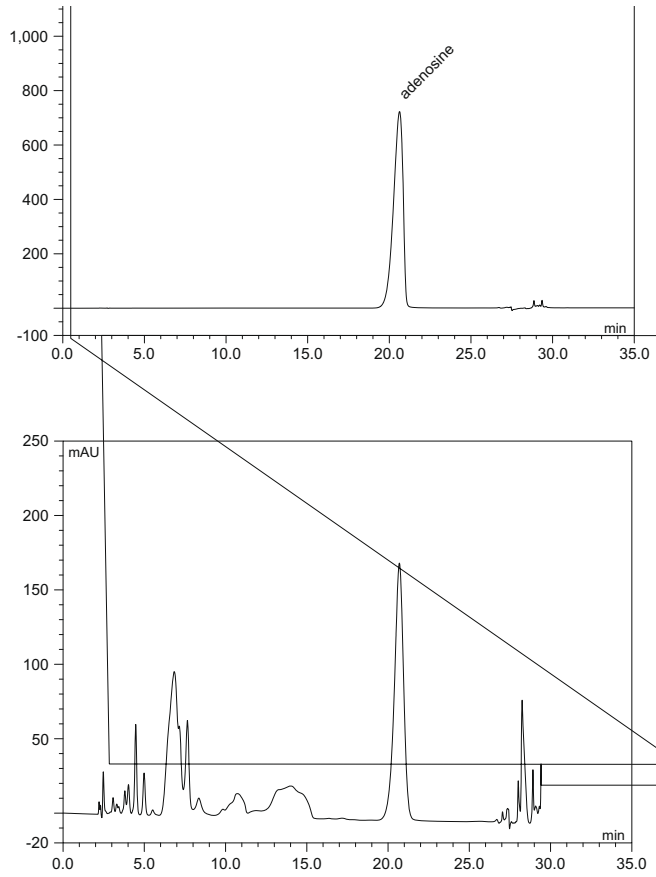
and 200 μg/ml. Both the stock and working solutions were kept at 4 °C for 1 month.

3. Results and discussion

3.1. Optimisation of sample extraction procedure

The water-soluble component of adenosine was extracted according to the previously developed procedures. However, these published extraction procedures were not suitable for RJ samples. There are many proteins (Crane, 1990; Palma, 1992; Piana, 1996a, 1996b) which should be removed from RJ samples before analysis. In a recently reported method, absolute ethanol was used that 80% ethanol not only precipitated the protein effectively but also gave satisfactory recovery.

Extraction with an ultrasonic processor is common practice for the exhaustive extraction of adenosine from various products for quantitative analysis. This extraction method was therefore used in this work. The effect of time on the extraction efficiency was evaluated by using 80% ethanol for 5, 15, 30, 60 and 120 min. The results are shown in Fig. 2: increasing the extraction time from 5 to 15 min resulted in an improved yield of adenosine; the extraction time was greater than 15 min (30, 60 and 120 min), the loss of adenosine was observed. In accordance with the above observations, the extraction procedure was carried out for duration of 15 min using 80% ethanol. At the same time, it is worth noting that



Since adenosine contains a polar ribose group, ionisation needs to be limited without compromising resolution; to achieve this gradient elution with a mobile phase containing acid is often used in reversed-phase liquid chromatography. In this study, the composition of the mobile phase was optimised by adding different acids (formic, acetic and phosphoric acids) to the aqueous phase to determine their effects on the enhancement of resolution, inhibition of adenosine ionisation and elimination of peak tailing of the target compounds. As a result, a mobile phase containing phosphoric acid was selected together with methanol which gave satisfactory resolution and a stable baseline. To improve separation selectivity and increase efficiency, different percentages of phosphoric acid were investigated. Finally, a mobile phase consisting of methanol and 0.4% phosphoric acid was chosen for the determination of adenosine in RJ. Typical HPLC–UV chromatograms of adenosine standard and actual samples are presented in Fig. 3.

The ultraviolet spectrum of the adenosine reference showed maximum absorbance values at around 204 and 257 nm. A stable baseline was found at around 257 nm. Thus, in this study, 257 nm was selected as the detection wavelength.

3.3. Stability test

Since adenosine can degrade to adenine (Kieszling et al., 2004) a stability test should be carried out. In this study we examined the

stability of adenosine in both the standard solutions and the actual RJ samples.

The stability of the adenosine standard solution maintained at room temperature was tested by repeated HPLC analysis of the same concentration (10 μg/ml) at different times during the day, and comparing the adenosine concentration with that in a freshly prepared standard solution. The results are shown in Table 1. No significant differences in adenosine concentration were found (RSD < 0.5%), indicating that the adenosine standard solutions were quite stable at room temperature.

The stability of adenosine in RJ samples was assessed by comparison of the results of repeated HPLC analysis of four samples stored for 2, 5, 10 and 24 h at room temperature containing known concentrations of adenosine. No significant decrease in adenosine concentration in the RJ samples was observed, thus indicating that the samples were stable for 24 h. The stability data are summarised in Table 1.

3.4. Linearity and calibration standards

The linearity of the method was calculated using various concentrations of adenosine (0.1, 1, 5, 10, 100 and 200 μg/ml) and repeating the experiments in triplicate. Calibration plots for adenosine yielded the linear relationship $y = (3.0303 \pm 0.0042)x - (0.8318 \pm 0.0039)$, where y and x are the peak area (mAU) and concentration of the standard solution (μg/ml), respectively. Linear regression showed good linearity in the range of 0.1–200 μg/ml with a correlation co-efficient of 0.9997. This allows the determination of adenosine over a wide range of concentrations.

3.5. Limit of detection and limit of quantification

Based on signal-to-noise ratios of 3 and 10, the limit of detection (LOD) and limit of quantification (LOQ) were determined using standard solutions of adenosine subjected to HPLC and analysed using the methodology described in Section 2.2. The LOD and LOQ were 0.017 and 0.048 μg/ml, respectively.

3.6. Recovery, accuracy and precision

Recovery was examined by adding a known amount of adenosine standard to RJ samples. The mixtures were extracted and analysed using the method described above. Table 2 shows the recoveries of adenosine from RJ samples.

The mean extraction recoveries of adenosine from RJ were found to range from 91.6% to 98.3%. The intra- and inter-day accuracy and precision values of the method are presented in Table 2. The co-efficient of variation of both inter- and intra-day analysis varied from 2.4% to 5.3%. These results show that the method is accurate and precise as evidenced by the high recovery and low CV values.

3.7. Application of the analysis to actual samples

The method was applied to actual samples collected from beekeepers and supermarkets. In total, 45 samples were analysed, and the adenosine content ranged from 5.9 to 2057.4 mg/kg. The details are summarised in Table 3. According to the results, some RJ samples were rich in adenosine, but there was a large variation in adenosine content amongst the samples. Time of harvest, storage conditions and the flower of origin likely account for the considerable variation of adenosine content in RJ samples.

4. Conclusions

In this study, a HPLC method has been established for the qualitative and quantitative analyses of adenosine in RJ. The method is simple, sensitive and reliable. From the results of analyses of a selection of actual RJ samples, adenosine was found to be relatively abundant in some products. Having in mind that adenosine plays a vital role in the activity of cells in living organisms, the method described here can be used to study the activity of adenosine in RJ. Comparing the results of the analyses, we found a substantial variation in the adenosine content of the RJ samples tested. It will be interesting in future research to investigate the factors led to the variation in the adenosine content of different RJ samples.

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