

## **Weakness of External Standard Calibration Curve for Quantification of Caffeine in Several Instant Coffee Brands by UV-VIS Spectrophotometer**

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### **Structured Abstract**

**Background:** Caffeine in coffee is a natural stimulant that can improve focus and energy level. Quantification of caffeine in instant coffee available in Malaysian market is important for the quality control purposes and to assist those who has low tolerance with caffeine to plan their daily coffee intake. In this work, UV-VIS spectrophotometer was used to measure the caffeine signal in the samples. The signal of caffeine measured by the UV-VIS spectrophotometer was extracted from the maximum absorbance value of the spectra. The instrument did not measure the concentration unit. Therefore, a calibration curve was plotted to obtain a relationship between the maximum absorbance and its corresponding concentration unit. In this work, an external standard calibration curve was explored for estimation of caffeine content in five brands of instant coffee.

**Methods:** For each sample brand, about 0.5 g of coffee was weighed and placed in a beaker. About 50 mL preboiled distilled water then added into the beaker before heated to 60 °C with presence of magnetic stirrer. After 10 min, the sample solution was filtered, and the residue was rinsed twice with 15 mL hot water. The filtrate was then extracted by liquid- liquid extraction, using the dichloromethane (DCM) as the extractant. The caffeine extracted in the DCM was added with 1 g anhydrous sodium sulfate to remove any trace of water. The DCM is removed by heated in water bath and then re-dissolve the sample solution with methanol. To construct external calibration curve, caffeine standard solutions with concentration between 5 and 50 mg/L were prepared in methanol. The caffeine spectra in sample and standard solutions were measured at range 200 – 350 nm.

**Results:** Caffeine content in Pendekar and Chek Hup were accurately quantified using the external calibration curve (% bias < 10 % when compared with the value manufacturer's claim). However, the method inaccurately quantified caffeine content in Aik Cheong, Yit Foh and Hang Tuah as these samples had matrix interference that cannot be corrected by the external calibration method.

**Conclusion:** External standard calibration curve can accurately quantified caffeine content in coffee samples, but the sample must not consist of any matrix interferences.

**Keywords:** caffeine, coffee, external standard calibration curve

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