



ZirChrom®

# Determination of Caffeine in Brewed Coffee by HPLC

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In this application we determined the caffeine content in coffee using a ZirChrom®-PBD analytical HPLC column and a ZirChrom®-PBD guard column.

### Introduction

Coffee is a very common caffeinated beverage that is produced by pouring hot water through ground coffee beans. The caffeine level in coffee can vary due to the brewing method used and the natural amount found in the bean. This method was developed to quickly determine caffeine content in brewed coffee using a very simple and green mobile phase via direct injection of an undiluted sample of brewed coffee by HPLC using a zirconia-based reversed-phase stationary phase (ZirChrom®-PBD) with a guard column.

### Experimental

The caffeine standard used was obtained from Sigma-Aldrich (catalog number C0750-5G). Standard stock solutions of 0.0052, 0.099, 0.145, 0.286, 0.472mg/ml were prepared, and 5 microliters of each solution were injected into the HPLC system. The peak area was then recorded to form the standard curve. Three batches of brewed coffee were analyzed by passing 10 ml of brewed coffee were filtered through a 0.45 micron syringe filter. The peak area was recorded and the caffeine content was quantitated. The following chromatographic conditions were used:

- Column: ZirChrom®-PBD, 150mm x 4.6mm, 3um (part # ZR03-1546)
- Guard Column: ZirChrom® PBD, 10mm x 4.0mm (Part#: ZR03-G40); guard holder (Part#: 850-00)
- Mobile Phase: 100% 5mM ammonium hydrogencarbonate and water solution
- Temperature: 50 °C
- Flow Rate: 1 ml/min.
- Detection: UV at 254 nm

Figure 1 shows a representative separation of the direct injection of brewed coffee. Figure 2 shows a representative standard curve for caffeine analysis based on peak area. The peak with a retention time of 4.358 min is caffeine as determined by the standard.

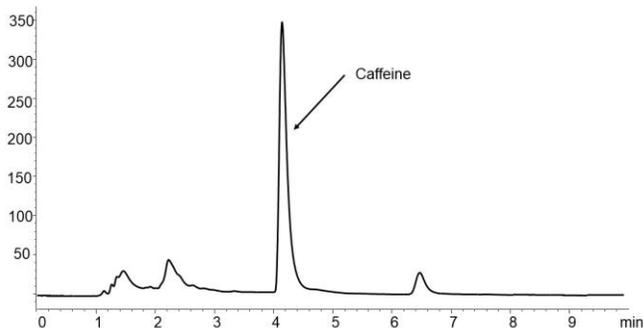


Figure 1: Representative chromatogram of the direct injection of brewed coffee on a ZirChrom®-PBD column with a 10mm ZirChrom®-PBD guard column.

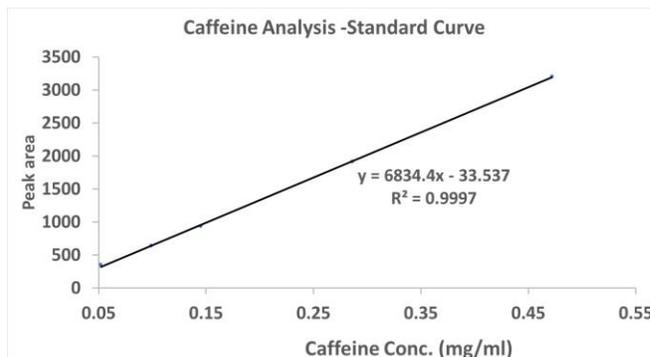


Figure 2: Caffeine standard curve.

Table 1 shows the quantitative results of the determination of caffeine in three batches of brewed coffee.

Batch Number of brewed coffees	Caffeine Concentration (mg/ml)	Caffeine Concentration (mg/cup) 1cup = 12 oz
1	0.53	187
2	0.53	187
3	0.54	191

Table 1: Results of caffeine determination.

The concentration of caffeine in brewed coffee is about 0.53 mg/ml and a cup has about 189 mg of caffeine. This method can be used for the rapid and direct determination of caffeine in brewed coffee.

ZirChrom technical support can help to optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or [support@zirchrom.com](mailto:support@zirchrom.com) for details.

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