

Full Length Research Paper

## Second derivative spectrophotometric determination of cyclophosphamide in pharmaceutical formulations

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**A new, simple, rapid, wide applicable range and reliable second derivative spectrophotometric method has been developed for determination of cyclophosphamide (CP) in bulk and pharmaceutical dosage forms. Calibration graph is linear in the concentration range of 25 - 200 µg/ml of CP with 10 µg/ml of detection limit and correlation coefficient of 0.9976. The precision and accuracy were acceptable depending upon the values of relative standard deviation and error percentage. Developed second derivative spectrophotometric method can be directly and easily applied for analyzing pure form and commercial pharmaceutical preparations of CP. The method was compared with a standard high performance liquid chromatography (HPLC) method and can be used for the quality control of pharmaceutical preparations in Erbil City.**

**Key words:** Cyclophosphamide, derivative spectrophotometry.

### INTRODUCTION

Cyclophosphamide (CP) is widely used in cancer chemotherapy, mostly in combination with other antineoplastic agents, and as an immunosuppressant (Karahalil and Akkoyunlu, 2003). Cyclophosphamide (belongs to the group of alkylating agents) is a pro-drug that is activated via 4-hydroxylation by cytochromeP450s such as CYP2B6 and CYP3A4 to generate alkylating nitrogen mustards (phosphoramidate mustard and the byproduct acrolein). The resultant mustards can alkylate DNA to form DNA-DNA cross-links, leading to inhibition of DNA synthesis and cell apoptosis (Malothu et al., 2009).

Several analytical methods have been reported and published for quantitative determination of CP in biological fluid and environmental samples, such as: High performance liquid chromatography (HPLC) methods

(Malothu et al., 2009; Ahmad et al., 2011), gas chromatography (GC)-mass spectrometry (Sugiura et al., 2010), and after extraction of CP by solid phase extraction techniques, using solid-phase extraction and GC-MS spectrometry (Martins et al., 2004), on-line sample preparation method by micro-extraction packed sorbent (MEPS) followed by LCMS/MS (Kamel et al., 2009), liquid chromatography with diode array detector (Alcântara et al., 2010), and spectrophotometric method (Karen et al., 2009). To our knowledge, there are no derivative spectrophotometric methods concerning the determination of cyclophosphamide in pharmaceutical formulations.

Derivative spectrophotometry is an analytical technique of great utility for extracting both qualitative and quanti

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**Abbreviations:** CP, Cyclophosphamide; HPLC, high performance liquid chromatography; GC, gas chromatography; MEPS, micro-extraction packed sorbent.

tative information from spectra composed of unresolved bands, and for eliminating the effect of baseline shifts and baseline tilts. It consists of calculating and plotting one of the mathematical derivatives of a spectral curve. Derivative spectrophotometry is now a reasonably prized standard feature of modern micro-computerized UV spectrophotometry (Patel et al., 2010). Derivative UV spectrophotometry has been widely used as a tool for quantitative and control analysis in agricultural, pharmaceutical and biomedical fields (Stanisz et al., 2009). The aim of this study was to develop a simple, rapid and efficient second derivative spectrophotometric method for the determination of CP in pharmaceutical formulations and compared with HPLC method that can be used for the quality control of pharmaceutical preparations in Erbil City.

## MATERIALS AND METHODS

### Apparatus

A CECIL CE 3021 UV/Vis scanning spectrophotometer equipped with 10 mm path length quartz cell was used to record normal and second derivative spectra of CP solutions.

### Chromatographic conditions

HPLC instrument, Smartline manager 5000, smartline UV detector 2500, smartline column thermostat 4000, smartline HPLC pump 1000. Knauer advanced scientific instruments, Germany with analytical column: C18, 5  $\mu$ m, 100 x 4.6 mm from Dr. Ing. H. Knauer GmbH, Germany. The mobile phase was acetonitrile: water (30:70) with a flow rate: 1 ml/min and injection volume 20  $\mu$ l. The eluent were monitored spectrophotometrically at 197 nm at temperature 30°C.

### Chemicals

All chemicals used were of analytical reagent grade. Stock CP solution (1000  $\mu$ g/ml) (Sigma-Aldrich) was prepared by dissolving 1000 mg of CP in distilled water and diluting to 1000 ml in a volumetric flask and stored in a refrigerator. Each working standard solution was freshly prepared by diluting the stock solution with distilled water.

### Recommended procedure

The standard solutions were prepared by dilution of the stock solution with distilled water in a concentration range of (25 - 200)  $\mu$ g/ml for normal and second derivative spectrophotometric methods. Distilled water was used as a reagent blank. The second derivative spectral recording were carried out using smoothing of 2 and 25 nm of interval at a scanning speed of 10 nm/sec in the wavelength range of 190 - 400 nm.

### Sample preparation

Amount of the CP vial content were accurately weighed and dissolved in distilled water and transferred to 25 ml volumetric flask, then diluted to the mark with distilled water. Appropriate dilutions

were made to obtain a solution in the concentration range of the calibration curve, and recommended procedure was applied.

## RESULTS AND DISCUSSION

The normal and second derivative spectra of the solutions of CP were recorded at the wavelength range of 190 - 400 nm against reagent blank as shown in Figures 1 and 2 which show maximum absorption at 193 nm for normal spectra and 207.3 nm for second derivative spectra of the solutions, respectively.

### Statistical data of the calibration curve

Under the recommended experimental conditions for second derivative spectrophotometric method the calibration graph was found to obey Beer's law in the concentration range of 25 - 200  $\mu$ g/ml, with the detection limit of 10.0  $\mu$ g/ml. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equation of calibration curve is  $y = 0.0456x + 0.0982$  and  $R^2 = 0.9976$ .

### Precision and accuracy

The precision and accuracy of the second derivative spectrophotometric determination of CP were studied depending upon the value of the relative standard deviation percentage (RSD %) and the relative error percentage (error %) for five replicate measurements of three different concentrations, respectively. Table 1 shows the results.

### Application of the method

The proposed method was applied to the determination of CP in some vials of CP samples from Baxter Company which are the only vials available in the hospitals of Erbil City. In the present method, from each vial samples (500 and 1000) mg different solutions were prepared and the recommended procedure was applied. The results are summarized in Table 2. The method was in a good agreement in comparison with the results obtained using HPLC for the standard CP (Figure 3) and qualification and determination of CP in the two vial samples as shown in Figures 4 and 5.

## Conclusion

The second derivative spectrophotometric methods have been described for quantification of cyclophosphamide in pure form of CP and its ratio in the vial content, at the time there were no any UV and derivative

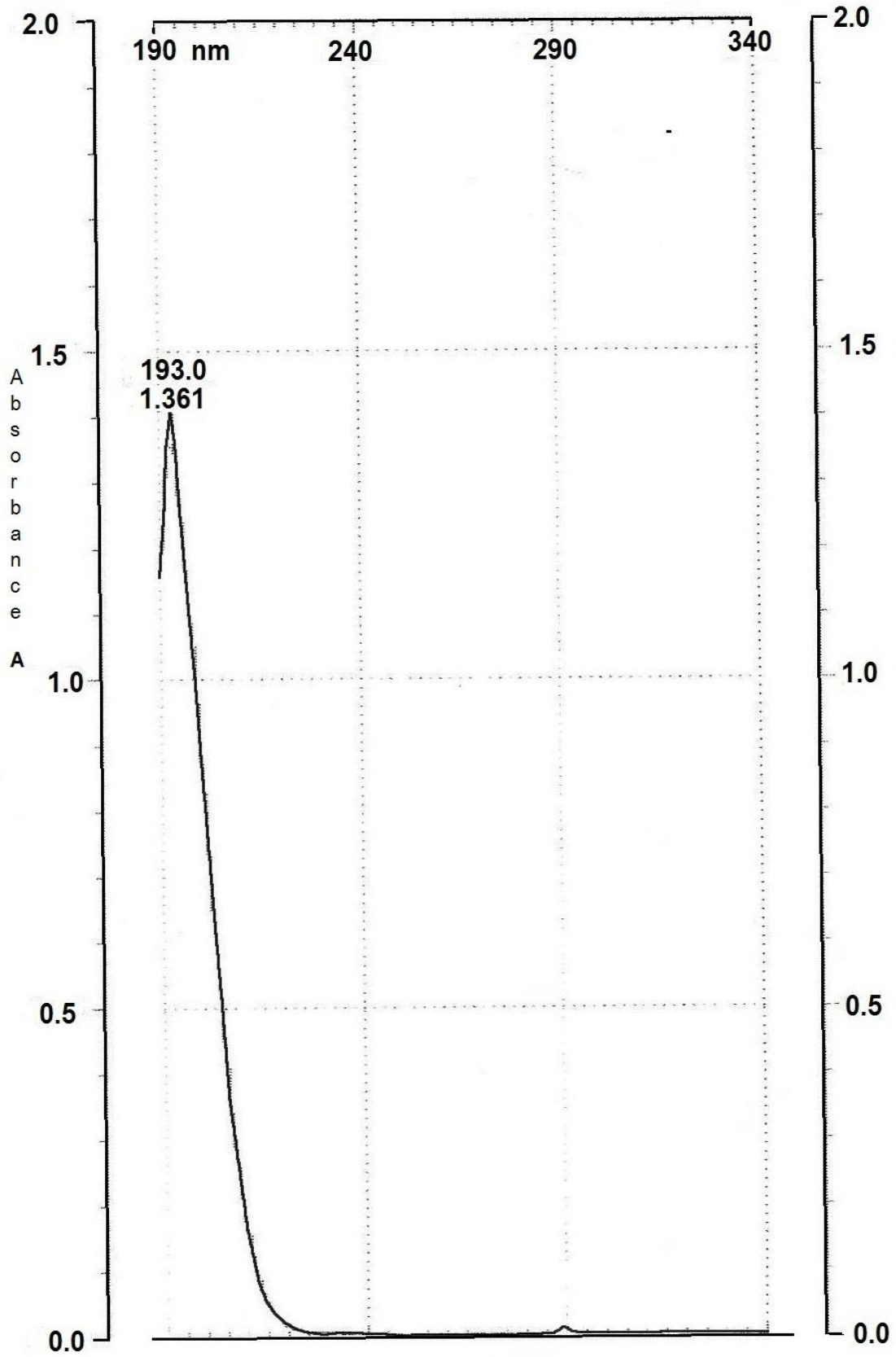


Figure 1. Normal spectrum of CP solution.

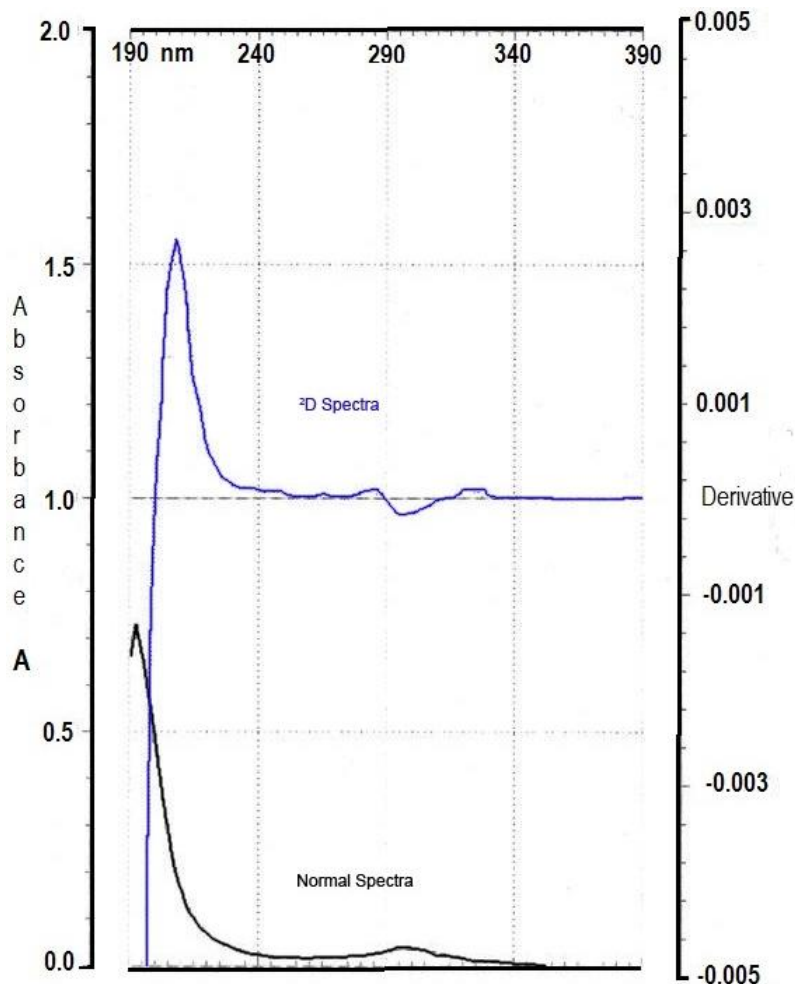


Figure 2. Normal and second derivative spectra of CP solution.

Table 1. Precision and accuracy of the proposed second derivative spectrophotometric method.

Cyclophosphamide concentration ( $\mu\text{g/ml}$ )	RSD%	Error%
25	2.19	+ 3.67
100	1.19	+ 1.79
200	0.75	+ 1.56

Table 2. Determination of CP in vial samples with the proposed method.

Samples	Amount found with proposed $^{2\text{D}}$ method (mg)	Amount found with modified HPLC method (mg)	Recovery with the proposed $^{2\text{D}}$ method %
500 mg	504.050	501.138	100.81
1000 mg	1009.040	1014.448	101.4

spectrophotometric methods for this aim. The proposed method is simple, rapid, wide applicable range, no requires extraction step and reagents to the determination of CP in the pharmaceutical formulations in

compare with other methods like HPLC. The method shows a good precision and accuracy and it is in a good agreement in comparison with standard HPLC method and can be used for the quality control of pharmaceutical

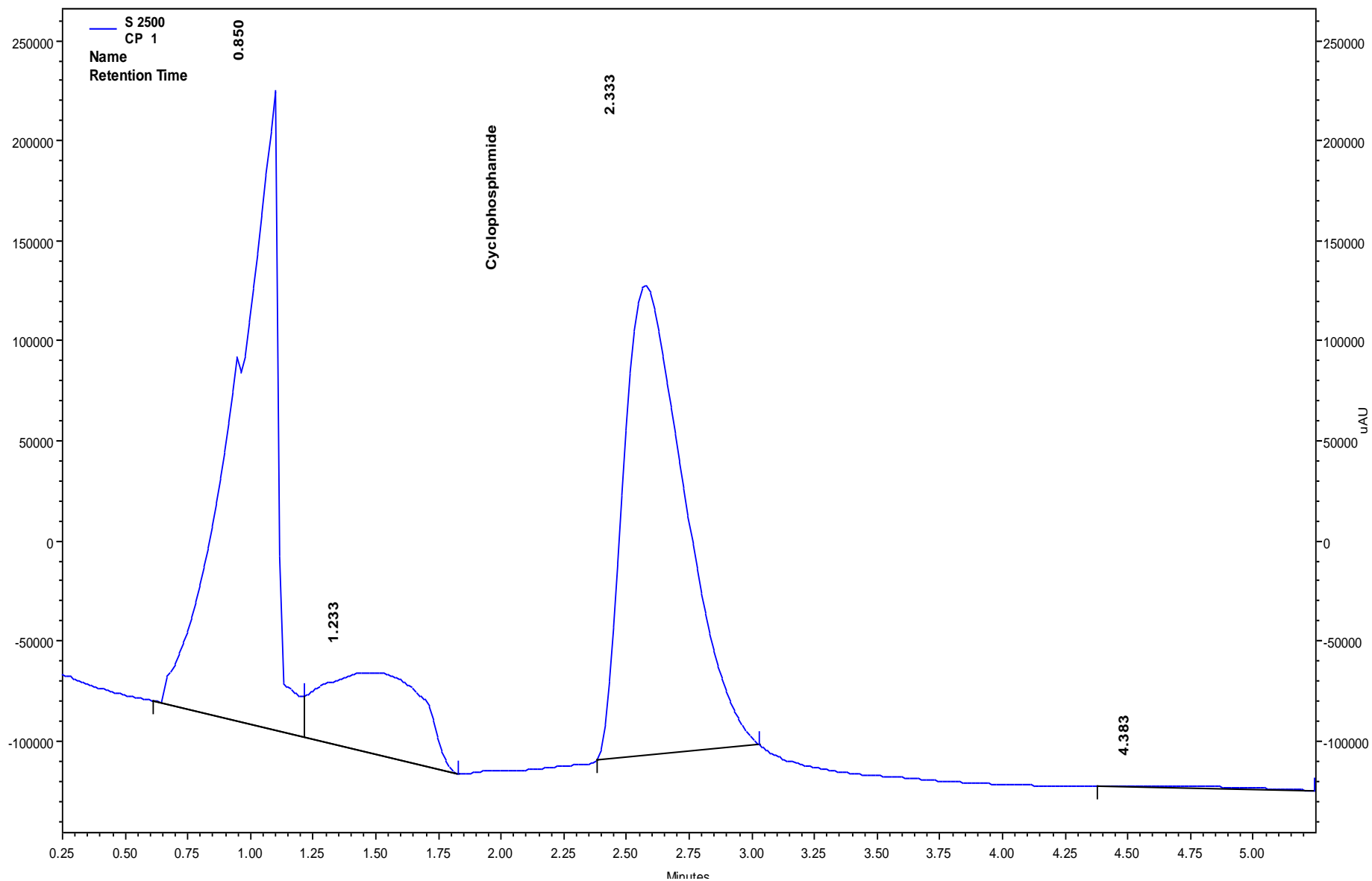


Figure 3. Representative chromatogram of cyclophosphamide.

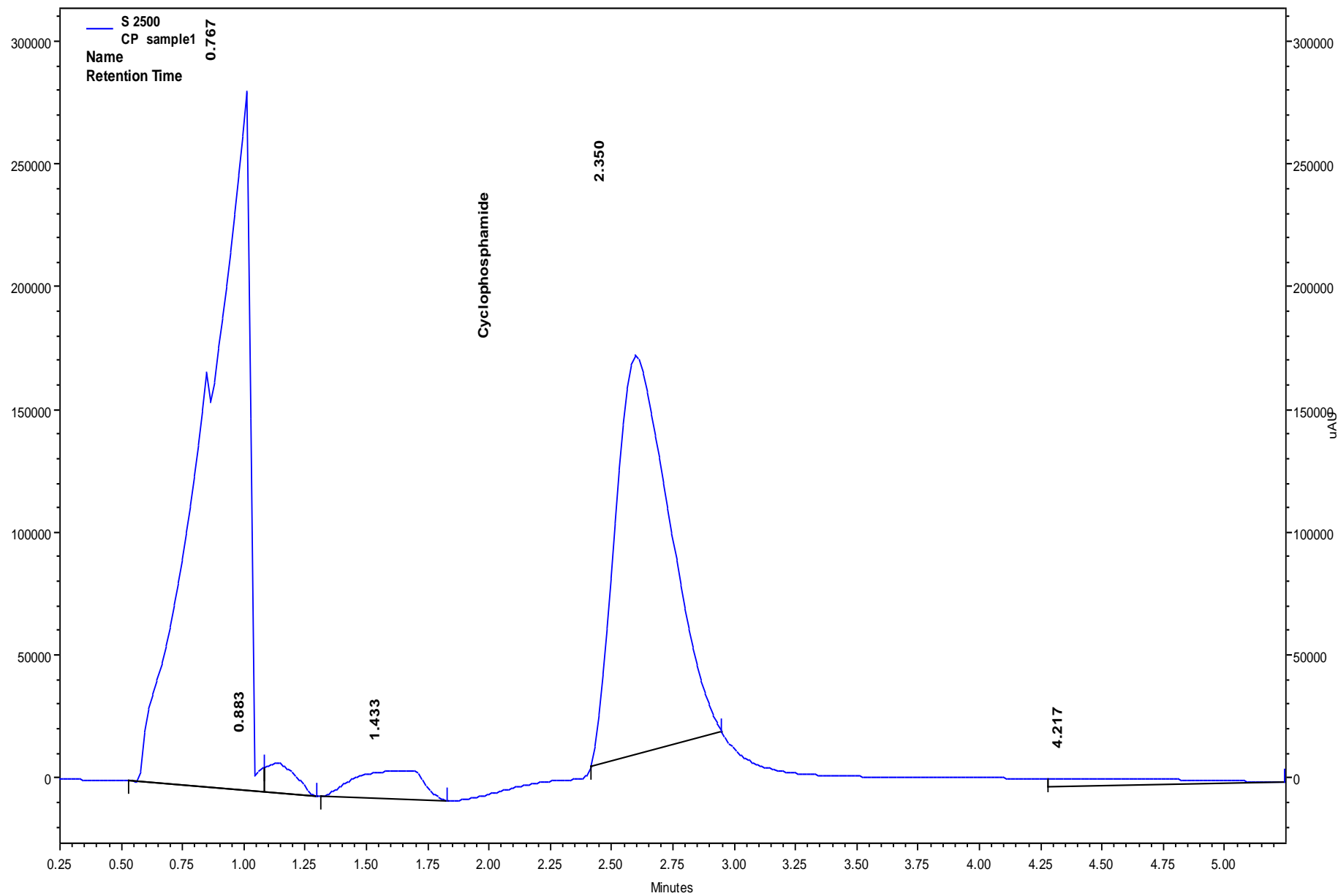


Figure 4. Chromatogram of the first sample.

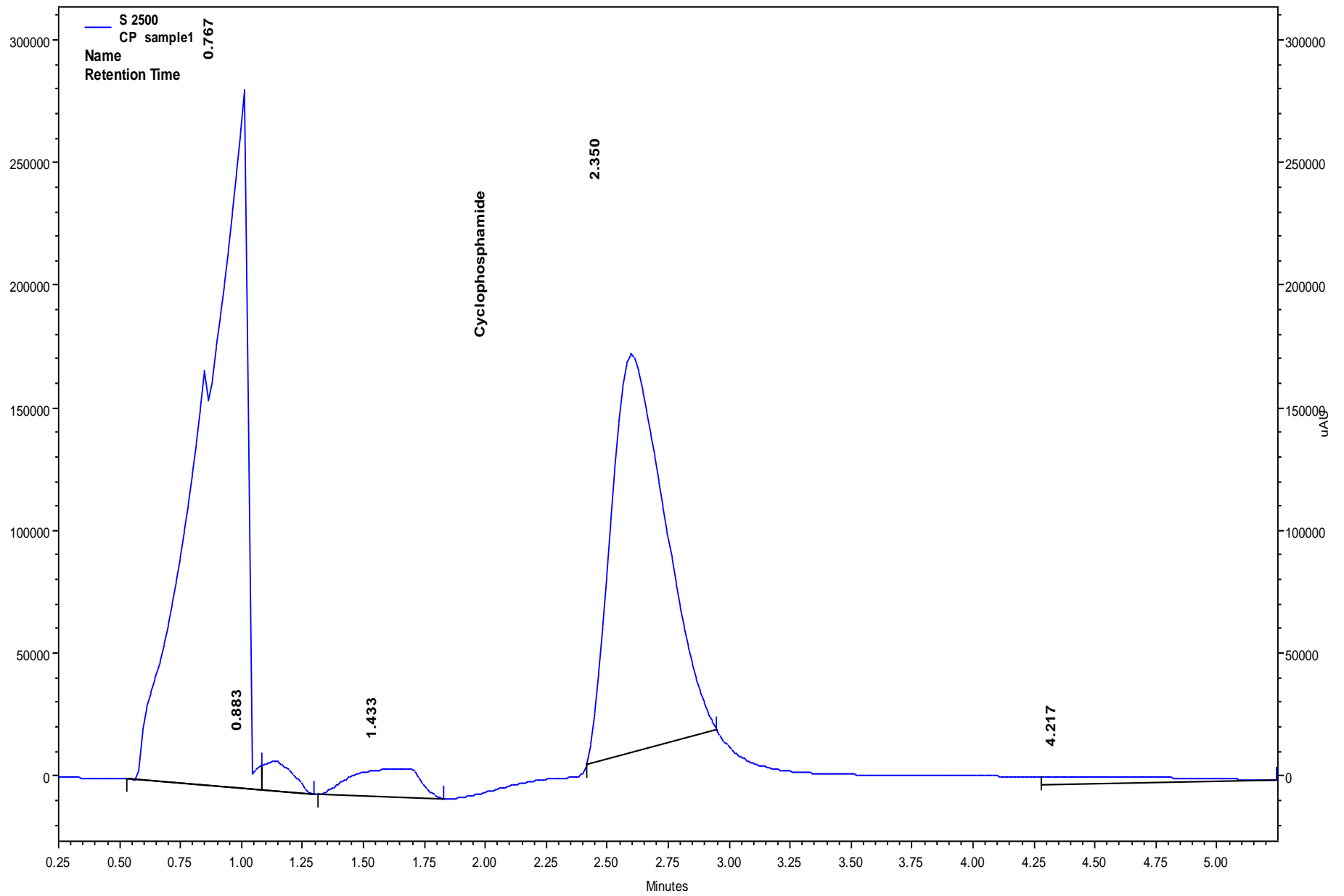


Figure 5. Chromatogram of the second sample.

preparations in Erbil City.

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