INTERNATIONAL DEVELOPMENT PLANNING REVIEW
ISSN:1474-6743 | E-ISSN:1478-3401

DESIGNING A FLOW INJECTION SYSTEM FOR THE DETERMINATION OF CAPTOPRIL DRUG USING DTNB

Raheem Kubaish Barid, Mohammed Turki Khathi
Department of Chemistry, College of Science, University of Thi–Qar, 64001, Thi-Qar, Iraq
Corresponding author: rah.ku.ch@sci.utq.edu.iq

Abstract
The stopped-flow injection technique is a simple analytical method with many applications, especially in chemical analysis based on the physical and chemical treatment of a diluted sample injected into the carrier stream. Its speed and high accuracy characterizes the technique and is cost-effective as it does not require high concentrations and large volumes of the substance to be estimated and the reagent used. Furthermore, it is an environmentally friendly technology, considered one of the most important chemical analysis techniques, including the determination of drugs in pharmaceutical preparations.
Captopril is a medication that includes L-proline, which enhances its bioavailability when taken orally. It has multiple applications, including treating high blood pressure, heart disease, and diabetic nephropathy, as well as preserving kidney function. Additionally, it has been used as an antidepressant.
In this technique, captopril is reacted with DTNB reagent in the presence of buffer solution (Na2HPO4) to produce a yellow product with a wavelength of 421 nm. The reaction is carried out under certain physical and chemical conditions, such as a flow rate of 1.0 ml/min, a reaction coil length of 100 cm, and a reaction time of 20 seconds. The technique can detect drug concentrations within the range of 1-200 ppm, with a limit of detection (LOD) and limit of quantitation (LOQ) of 0.22 ppm and 0.764 ppm, respectively. The technique has a relative standard deviation (RSD) 568 and a correlation coefficient (R2) 0.9984.
Keywords: flow injection, DTNB, Captopril.

1. Introduction

A Continuous Flow Injection Analysis (CFIA) manifold is a device that injects a liquid sample into a continuous carrier stream of a suitable liquid using a CFIA manifold without any gaps between the sample and the carrier stream. This equipment is simple, affordable, and has a high sample rate and dependability while still being easy to use. The residence time of the sample in a non-segmented stream is much less than the residence time in an air-segmented system(1). Over the years, several other highly competitive flow injection instruments have been created and deployed to various applications, including traditional Normal Flow Injection (nFIA) injection analysis, which resulted in the emergence of several forms of this technique, including injection and stopped-flow injection analysis (rFIA), stopped-flow and confluent areas, Merging zone and others(2).

Johnson and Petty proposed this technique in 1982 as an early attempt to detect the amount of phosphate in seawater by injecting the reagent into the water(3). RFIA was utilized, which is a reversed FIA technique. The sample was pumped continuously rather than through a carrier and a premixed reagent was injected instead of a page. This is because dispersion in rFIA is lower than in traditional FIA, which reduces the diluting impact and increases insensitivity. Several factors significantly affect the phenomenon of dispersion(4). Some factors lead to an increase in the phenomenon of dispersion, which is the biggest problem that the chemical reaction suffers from.
The instrument used to introduce a specified and constant model size into the current of the carrier current injection is also called a rotary valve, hex valve, or hex valve, as the load current passes continuously in the bypass. The sample is temporarily loaded with bearing couplings of known lengths and diameters when injected by the syringe and then transferred to the injection site(5). The injected sample reacts with the reagents in the manifold.
Flow injection analysis is a versatile instrumental tool due to its simplicity, small sample volumes, low reagent consumption, and high reproducibility. Such analysis generally allows the acquisition of analytical data at a low cost and in a relatively short analysis(6). Captopril reacts with DTNB to form a yellow product, as shown in **Figure 1**.

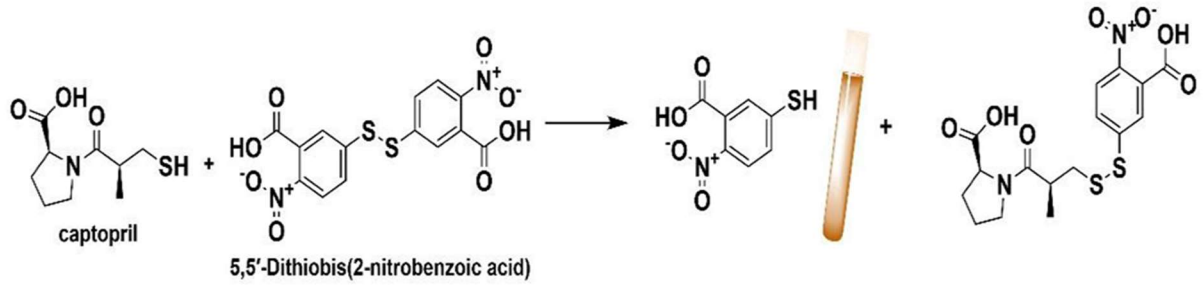


Figure 1. The reaction between Captopril and DTNB.

2. Materials and Methods

2.1. Chemicals All chemicals and reagents used in this project are analytical-grade materials [DTNB (Thomas Baker (Chemicals) Pvt. Ltd.), Na_2HPO_4 (Thomas Baker (Chemicals) Pvt. Ltd.), Captopril (Thomas Baker (Chemicals) Pvt. Ltd.), and Sodium Citrate (Thomas Baker (Chemicals) Pvt. Ltd.)].

2.2. Instrument

Several devices were utilized: Peristaltic pump, Rotary 6-port injection valve, Competence Analytical Balance, and Biologic Quad Tec UV-Vis Detector..

2.3. Sample preparation of Captopril 300 ppm:

The standard drug was prepared by dissolving 25 mg of Captopril in 25 mL of distilled water, then diluting the solution to 100 mL in a volumetric flask with distilled water..

2.4. Na_2HPO_4 salt 2000 ppm:

The material is dissolved in 100 ml to obtain a concentration of 2000 ppm after weighing 200 mg (0.2 g).

3. Results and discussion

3.1. Determination of Captopril in terms of DTNB using Flow Injection Analysis

We carefully selected the Accumulate Peak Analysis (APA) technique for its precise and speedy analysis, as well as its ability to clearly visualize data. The data we obtained through this method were compared to a standard solution using a specialized equation. By doing this, we were able to gain a deeper understanding of the factors that impact analysis performance, which will help us in the next stage. For further information, see Figure 2.

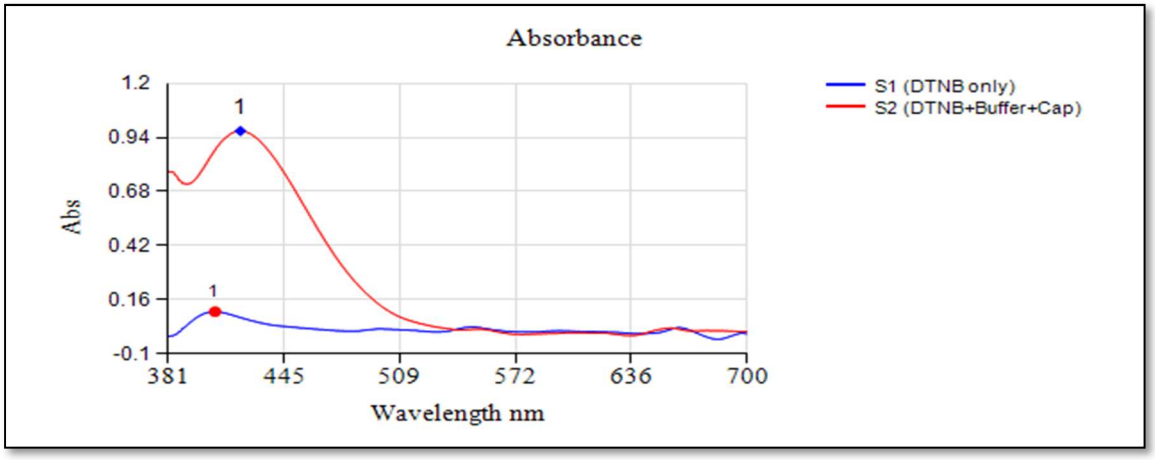


Fig [2]: UV-absorbance scanning spectrum for the reaction product between 10 ppm Captopril and 10 ppm DTNB with Na_2HPO_4.

3.2. Determine the Time of the stop

The experiment was conducted to determine and test the stability of the reaction and its peak at a time starting from 0, 10, 20, 30, 40, 50, and 60 seconds under the following conditions(7). The experiment was conducted to determine and test the stability of the reaction, as shown in **Fig [3]**.

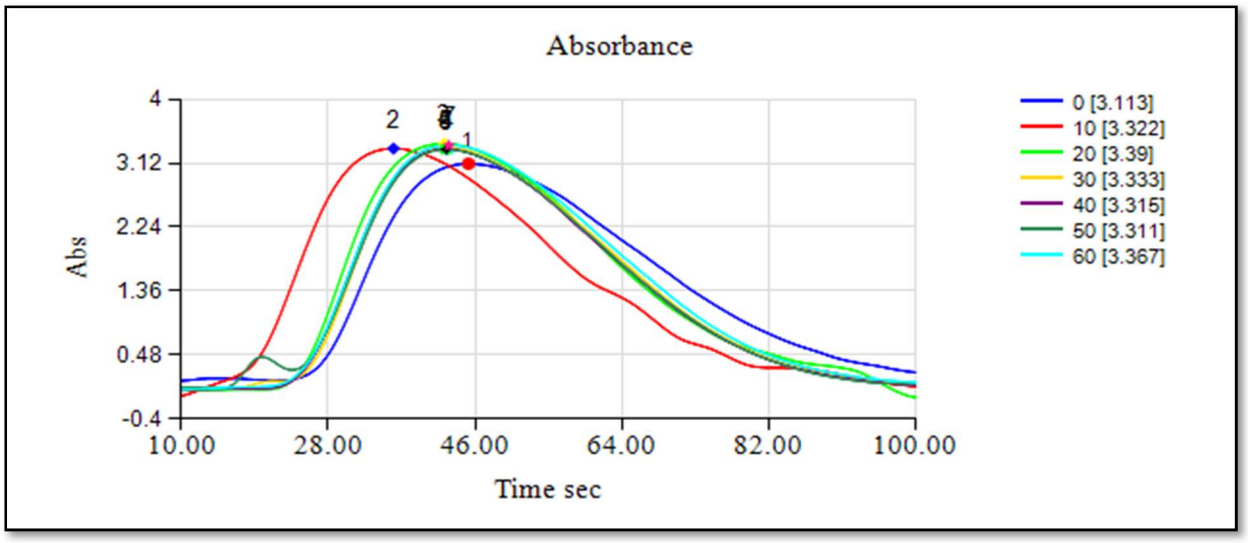
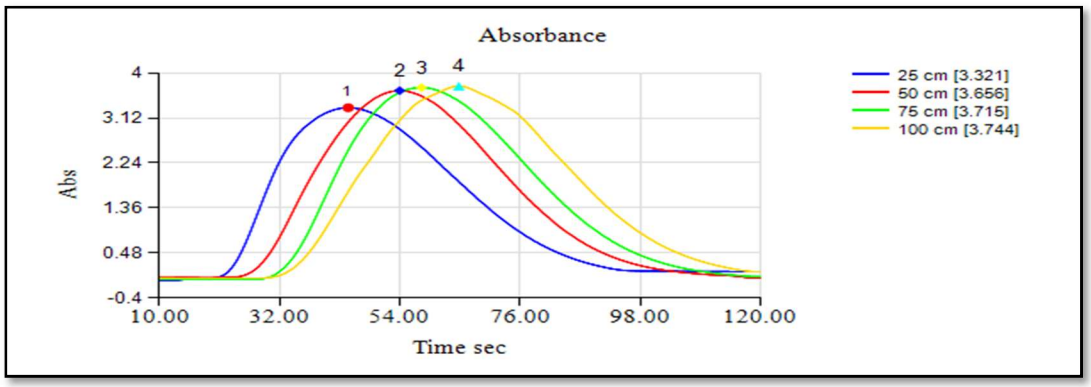


Fig [3]: Effect of time on the reaction of captopril with the reagent DTNB at wavelength

In order to determine pure ranitidine in pharmaceutical preparations, a stability time of 20 minutes is required for the source (12). An experiment was conducted to find the most effective reaction coil that would allow the reaction to reach the highest level of mixing. The experiment was conducted using reaction coils of varying lengths, starting from 25cm and increasing incrementally to 50cm, 75cm, and finally 100cm. The experiment was conducted under specific conditions:

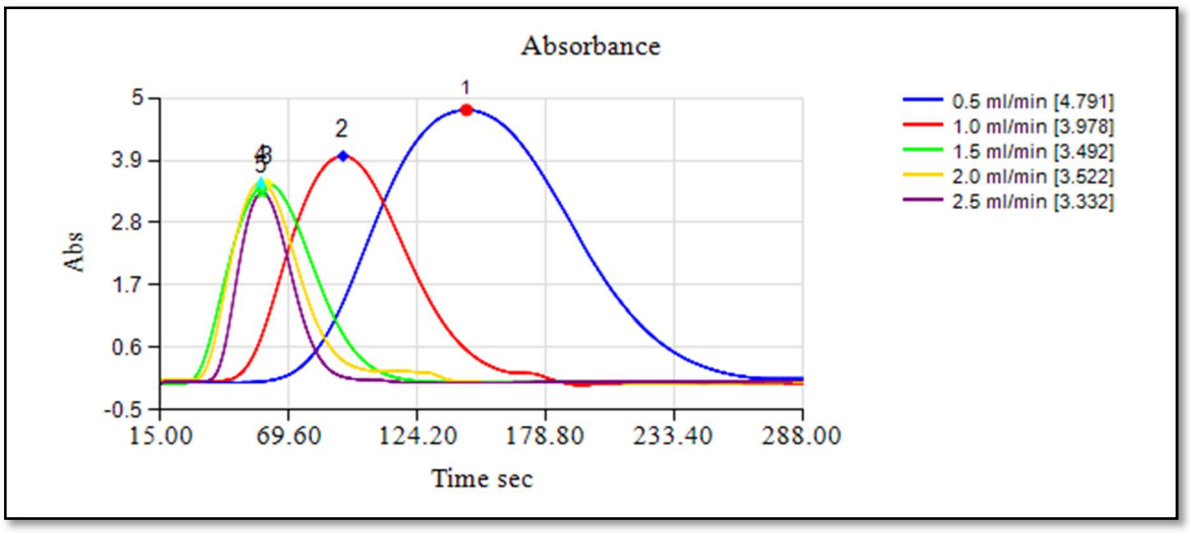
Drug



concentration =100ppm, reagent concentration 50ppm, Na_2HPO_4=2000 ppm, flow rate= 1.5 ml/min, the stopping time =20 seconds, and the wavelength is 421 nm at room temperature**,** as shown in the **Fig [4]**

Fig [4]: The absorption peak height was determined by the reaction coil under the following conditions: captopril conc. = 100 ppm, DTNB conc. = 50 ppm, Na2HPO4 = 2000 ppm, and at room temperature.

3.3. Determine the appropriate Flow Rate:During the experiment, the goal was to find out the



best flow rate for effective mixing without compromising response sensitivity. To achieve this, various pump speeds ranging from 0.5 mL/min to 2.5 mL/min were utilized. The experimental conditions are depicted in Figure 5.

Fig [5]: The effect of flow rate on the absorption peak height when captopril.

3.4. The effect of temperature on the interaction of the captopril with DTNB.

To determine the ideal temperature for the reaction without any breakage or disintegration, the experiment was conducted at different temperatures ranging from 30 to 60 degrees Celsius. The experiment was conducted under the following conditions: cap = 100 ppm, DTNB = 50 ppm, Na2HPO4 = 2000 ppm, wavelength = 421, using the reaction coil = 100 cm, with a stop time of 20 seconds and a flow rate of 1.0 ml/min(8). The findings are illustrated in Figure 6

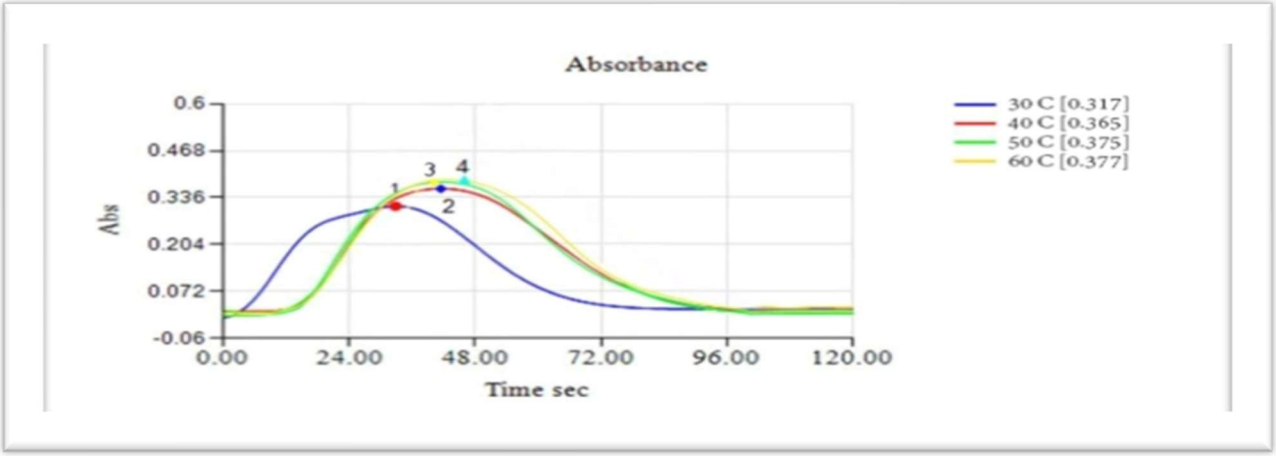


Fig [6]: Effect of temperature on the reaction of Cap and DTNB in the presence of Na_2HPO_4.

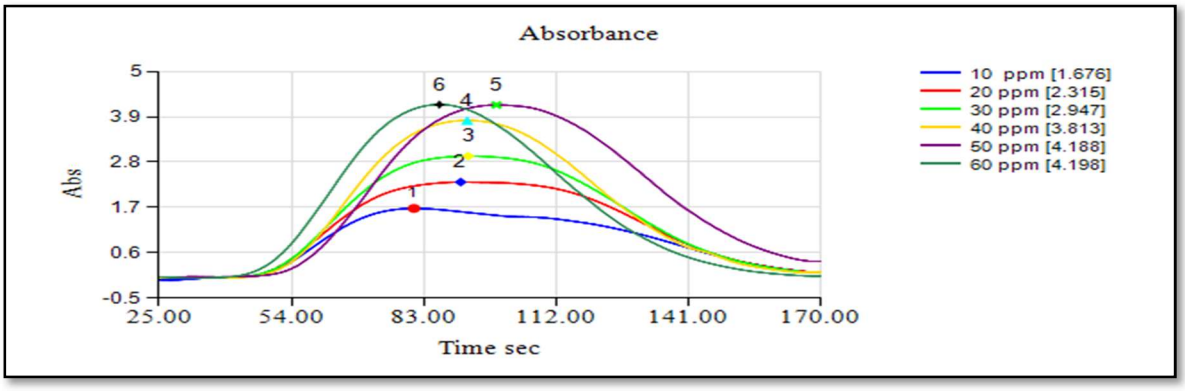
3.5. Effect of DTNB concentration:

The purpose of the experiment was to determine the optimal concentration of the reagent required for complete reaction. To achieve this, various concentrations of the reagent were tested, ranging from 10-60 ppm, under the following conditions:

Drug concentration was 100 ppm, Na2HPO4 was 2000 ppm, stop time was 20 seconds, the reaction coil was 100 cm, the flow rate was 1.0 ml/min, the wavelength was measured at 421 nm and the temperature was 40°C. Data is presented in Fig [7].

Fig [7]: The influence of DTNB concentration on the absorption peak height when captopril conc. = 100 ppm, reaction coil length = 100 cm, temperature=40 C^0, flow rate =1.0 ml/min, stop time =20 sec.

3.2.1. B.7. Effect of Na_2HPO_4 concentration:



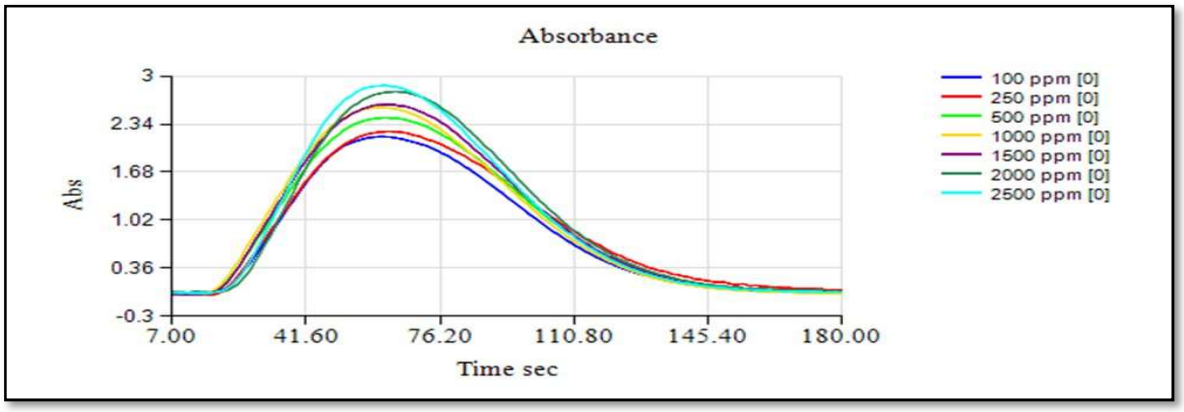


Fig [8]: The absorption peak height was studied with varying Na_2HPO_4 concentrations.

Experimental conditions included Cap conc. at 100 ppm, DTNB at 50 ppm, reaction coil length at 100 cm, the temperature at 40 C, flow rate at 1.0 ml/min and stop time at 20 sec.
Based on the obtained results, it was observed that the permeation increased with the concentration of the buffer until it reached a stable state. The absorption and response were found to be high at a buffer concentration of 2500 ppm, as it produced a clear peak without any distortion in comparison to lower concentrations. Therefore, it can be inferred that the optimal buffer concentration is 2500 ppm(9), as represented in Figure 8.

General procedures and the calibration curve are discussed below:
The quantitative determination of captopril was carried out under optimum conditions. To prepare the calibration curve, captopril solutions with varying concentrations were analyzed using the FIA system, and the absorption peak height value of the formed complex was plotted against the concentration at 421 nm. Using this method, captopril can be determined in the range of 1 to 200 ppm, as demonstrated in Fig [9] and Fig [10].

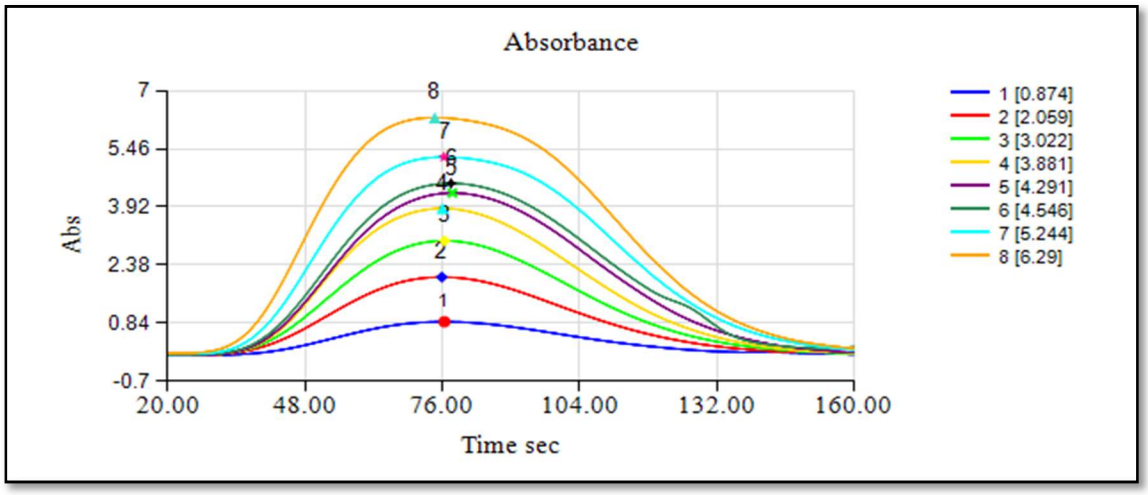


Fig [9]: Absorption spectra of Captopril.

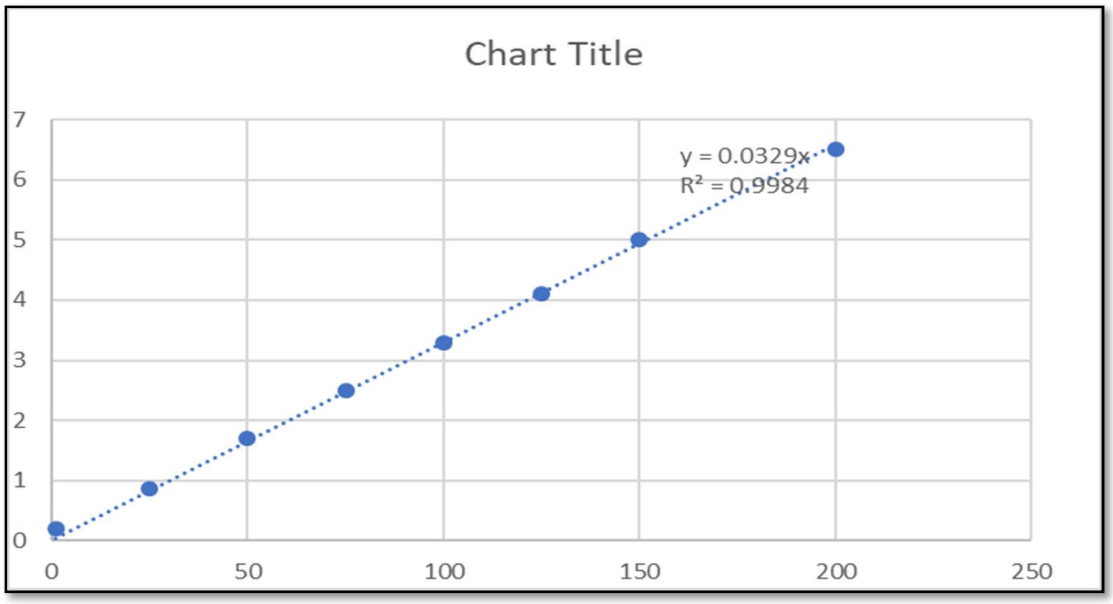


Fig [10]: Calibration curve of captopril.

The suggested method's sensitivity, represented by the detection limit (LOD) and quantitation limit (LOQ), has been estimated to be 0.22 ppm and 0.764 ppm, respectively. The standard deviation is 0.5242.

Table [1]: The effect of captopril conc. On the absorption peak height when DTNB conc. = 50 ppm, reaction coil length = 100 cm, Na_2HPO_4= 2500 ppm temperature=40 °C.

Index	Sample	cons. ppm	Peak Height
1	1	1	0.212
2	25	25	0.874
3	50	50	1.7
4	75	75	2.6
5	100	100	3.3
6	125	125	4.1
7	150	150	5.0
8	200	200	6.5

3.6. The method to determine captopril in water-based solutions is as follows:

Four aqueous solutions of unknown concentration were prepared, and their absorbance was measured under optimal conditions. The concentration of the solutions was then determined by calculating the absorbance of the drug concentration in the calibration curve and comparing it with the absorbance of the prepared drug concentration. Considering any preparation errors, the calibration curve provided values very close to the prepared solutions. This is illustrated in Figure 11.

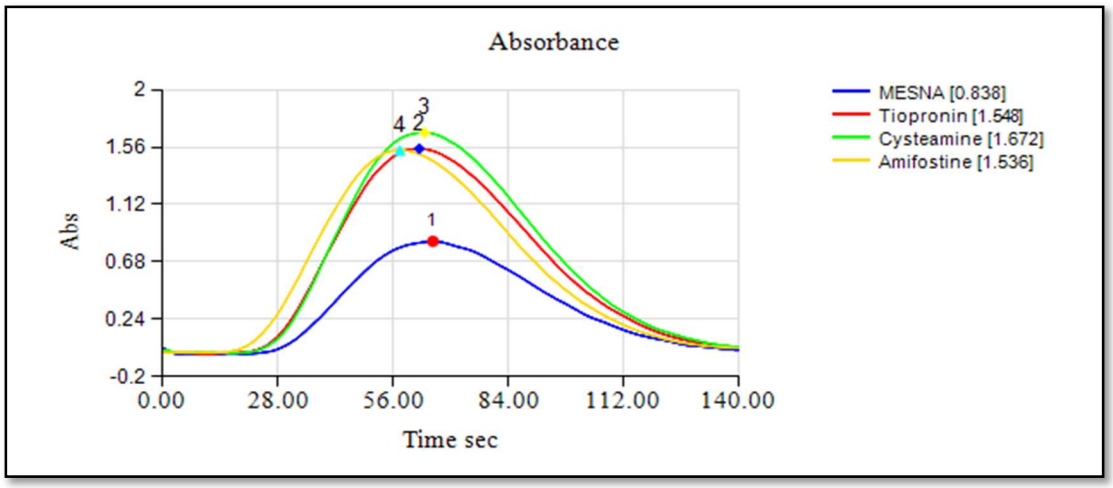


Fig [11]: The spectrum of possible applications.

Table 2: Value of sample application

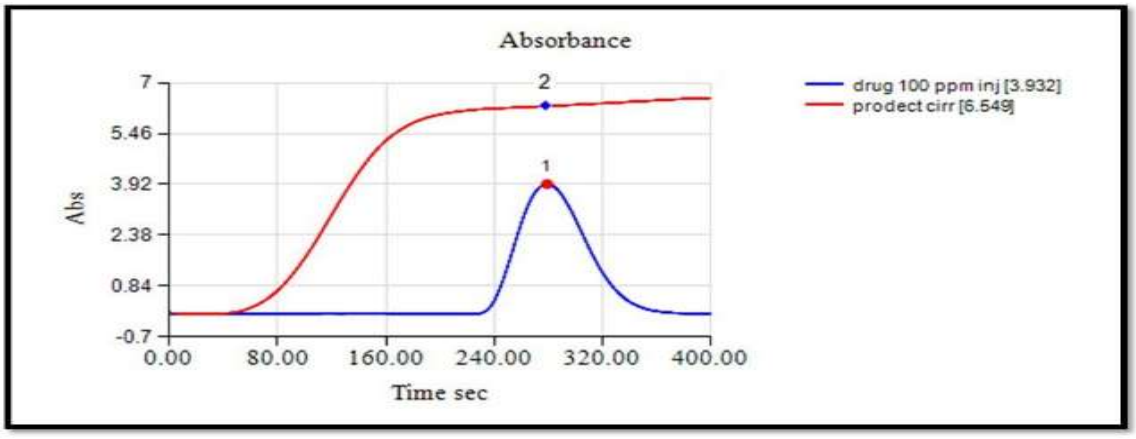
Sample	Peak Height	Taken (Sample)	Founded
MESNA	0.838	25	23.97
Tiopronin	1.548	50	45.52
Cysteamine	1.672	50	49.17
Amifostine	1.536	50	45.17

3.7. The determination of dispersion:

In order to demonstrate the extent of the dispersion process within the flow injection system from the injection point at the valve to the detecting point at the detector, the dispersion coefficient (dilution factor D) was studied. The experiment aimed to calculate the dispersion in the reaction, and two experiments were conducted for this purpose(11).

In the first experiment, the reagent and buffer were combined as a carrier solution and pumped through the valve with the drug (100ppm), resulting in an absorbance of 3.932A.

In the second experiment, A mixture of each drug at 100ppm, DTNB at 50ppm, and Na2HPO4 at 2500ppm was combined externally and used as a carrier solution without the use of an injection

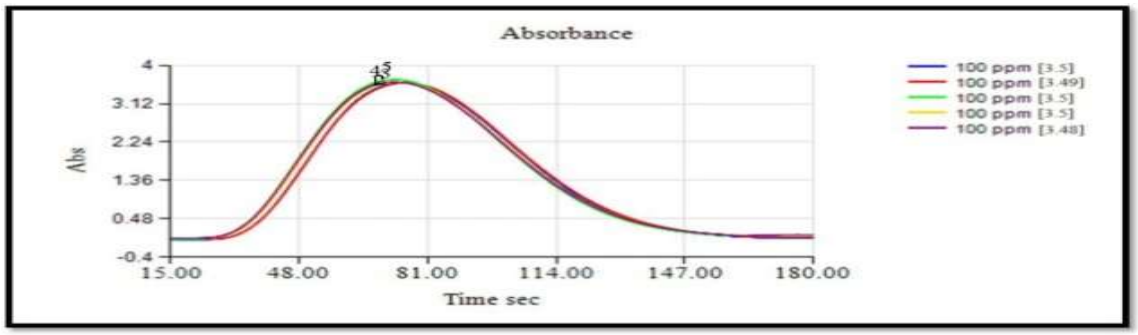


process. The absorbance was measured and found to be 6.549A. Based on these results, the dispersion can be calculated as 1.66, as shown in Figure 12.

Fig [12]: Dispersion coefficient study results for captopril determination FIA system.

Repeatability:

In order to determine the accuracy and sensitivity of the proposed method, an experiment was conducted. The absorbance was measured five times in a row, with the drug concentration set at 100ppm and under optimal conditions. The aim was to measure the relative standard deviation (RSD%), as illustrated in Figure 13**Fig [13]: Repeatability of 100 ppm for captopril solution by FIA**It was found that the proposed method had high sensitivity and accuracy, with a standard deviation (SD) of 0.5242 and a relative standard deviation (RSD%) of 15.0%. **Table [3]: Repeatability results for 100 ppm captopril solution by FIA.**



Sample No.	1	2	3	4	5	Mean	SD	RSD%
Flu.	3.5	3.49	3.5	3.5	3.48	3.494	0. 5242	15.0%

Table [4]: different methods used to determine Captopril

Reagent	**λ_{max} nm**	**Technology**	**LOD** mg/L	**LOQ** mg/L	**R^2**	**Range** mg/L	**RSD%**	**Recovery %**	**Ref**
2-bromo-2X -acetonaphthone	246	Sensitive HPLC		10	0.998	12.5–500		95%	87
c&urn(W) in sulphuricacid medium.		chemilumin escence	0.037		0.998	0.1-6.0		95%	88

2-Chloro-1-methyl quinoliniumtetrafluoroborate	355	HPLC	0.05	0.1	0.999	0.1-200	0.15		89
ethyl-propiolate	285	HPLC	10	35	0.999	0.5–25.0		96%	90
Fe(III)/Fe(II)		Flow injection	0.012		0.999	0.03–3.6	0.97	101.8	91
Paracetamol	220	RP-HPLC-DAD	5.10×10^{-4}	1.56×10^{-3}	0.999	0.5-200	<2	99.45%–102.55%	92

. Conclusions

The flow injection technique can be used to identify drugs in the presence of reagents with thiol groups. In a recent study, all designed systems were found to be highly effective. This analytical method is known for its speed, accuracy, sensitivity, and responsiveness in detecting and estimating drugs with even the smallest concentrations, the largest number of samples, and the smallest volumes of reagents, all in a very short time. The flow injection system is also known for using tiny volumes, low concentrations of the reagent and the carrier solution mixture, and the small sample volume, thus minimizing chemical consumption. The new systems have high selectivity and recovery, good repeatability, fair relative standard deviation values, high sampling rate, precision, and accuracy. The calibration graph can also determine the linear range for the reaction of the DTNB reagent with Captopril in aqueous solutions.

References

1. El-Beshbishy HA, Tork OM, El-Bab MF, Autifi MA. Antioxidant and antiapoptotic effects of green tea polyphenols against azathioprine-induced liver injury in rats. Pathophysiology. 2011;18(2):125-35.
2. Hesham A, Autifi MA, Mariee AD. Green tea (Camellia sinensis) extract protects against azathioprine-induced hepatotoxicity and neutrophil infiltration in rats. Asian Journal of Traditional Medicines. 2010;5:1-11.
3. Perrett D, Rudge SR. The determination of thiols and related compounds using high-performance liquid chromatography. Journal of pharmaceutical and biomedical analysis. 1985;3(1):3-27.
4. Prithwish N. Mitigation of Azathioprine induced Oxidative hepatic injury by the Flavonoid quercetin in wistar rats: Vel's College of Pharmacy, Chennai; 2008.
5. Aleisa AM, Al-Rejaie SS, Abuohashish HM, Ola MS, Parmar MY, Ahmed MM. Pretreatment of Gymnema sylvestre revealed the protection against acetic acid-induced ulcerative colitis in rats. BMC complementary and alternative medicine. 2014;14:1-11.

6. Hamza AA, Heeba GH, Hassanin SO, Elwy HM, Bekhit AA, Amin A. Hibiscus-cisplatin combination treatment decreases liver toxicity in rats while increasing toxicity in lung cancer cells via oxidative stress-apoptosis pathway. Biomedicine & Pharmacotherapy. 2023;165:115148.
7. Rashid K, Wachira FN, Nyariki JN, Isaac AO. Kenyan purple tea anthocyanins and coenzyme-Q10 ameliorate post-treatment reactive encephalopathy associated with cerebral human African trypanosomiasis in murine model. Parasitology international. 2014;63(2):417-26.
8. Yeşilaltay A, Ersoy Ö, Omurtag G, Yurdun T. Effects of carbamazepine on hepatic glutathione level in rats and determination of carbamazepine and its epoxide metabolite in plasma by HPLC. Drug metabolism and drug interactions. 1998;14(4):251-8.
9. El-Hadary AE, Elsanhoty RM, Ramadan MF. In vivo protective effect of Rosmarinus officinalis oil against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. PharmaNutrition. 2019;9:100151.
10. Packialakshmi S. Immunomodulatory Activity of Ethanolic Extract of Propolis (Apismellifera Linn) on Cyclophosphamide Treated Immunosuppressed Rats: KM College of Pharmacy, Madurai; 2017.
11. Llewellyn DH. Sulphydryl-dependent reactions of D-penicillamine: Pharmacokinetics and effects on monocyte function in rheumatoid arthritis: University of Bath (United Kingdom); 1985.
12. Spectrophotometric Determination of Ranitidine-HCl in Pure Form and Pharmaceutical Formulations with Metol and Potassium hexacyanoferrate (III). (2019). University of Thi-Qar Journal of Science. https://doi.org/10.32792/utq/utjsci/vol4/3/25.