

Research Article

Spectrofluorometric Determination of Lisinopril dihydrate and Methyl- Dopa in Bulk and Pharmaceutical Formulation by Using Dansyl Chloride

Sobhy M. El-Adl¹, Mohamed El.Hossiny El-Sadek¹, Nariman M. Saeed^{2*}

¹Department of Medicinal Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

²Pharmacist, Ministry of Health, Egypt

*Corresponding Author: Nariman M. Saeed, Pharmacist, Ministry of Health, Egypt, Tel: (20-2) 7951821; E-mail: nariman_saeed@yahoo.com

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Abstract

A novel spectrofluorometric method has been developed for quantitative determination of Lisinopril dihydrate and methyl-dopa either in pure form or in pharmaceutical preparation. The principle of the method based on the reaction of each drug with dansyl chloride [5-(Dimethyl Amino) Naphthalene-1-Sulfonyl chloride] by the aid of sodium hydroxide solution of pH 8.5 resulted in a product of a high fluorescent intensity which was then measured at 511 nm and 516 nm, for lisinopril and methyl-dopa respectively after excitation at 323 nm. Factors affecting reaction development were studied and optimized. The proposed method was found to be sensitive, selective and reproducible. Calibration curves were linear over the concentration ranges of 3–20 for lisinopril and 7–25 µg/mL for methyl-dopa. Results obtained from tablets analysis were compared statistically with reported reference methods.

Keywords: Spectrofluorometric; Dansyl chloride; Lisinopril; Methyl-dopa

1. Introduction

Lisinopril dihydrate [LIS] (S)-1-[N2-(1-carboxy-3-phenylpropyl)-lysyl-proline dihydrate (Figure 1). It has a molecular formula of $C_{21}H_{31}N_3O_5 \cdot 2H_2O$ and a molecular weight of 441.52 g/mol, official in BP and USP [1-2]. Lisinopril is non-sulfhydryl long-acting angiotensin converting enzyme inhibitor used as antihypertensive agent, prophylactic after myocardial infarction, it exerts a haemodynamic action and allows natriuresis, it was also found to be useful in preventing diabetic retinopathy [3-4]. Several analytical methods, including spectrophotometric methods [5-7], spectrofluorometric [8-10], chromatographic methods [11-14], polarographic methods [15] and titrimetric method [16] have already been reported for its determination alone or in combination with other drugs. Methyl-dopa [MD] L-3-(3, 4- Dihydroxyphenyl)-2-methylalanine sesquihydrate (Figure 1), it is a catechol derivative with a molecular formula of $C_{10}H_{13}NO_4 \cdot 1 \frac{1}{2} H_2O$ and a molecular weight of 238.24 g/mol, official in USP

[2]. "It is the drug of choice for hypertension in pregnancy". It acts centrally on alpha2-adrenoreceptor leading to a decrease in sympathetic tone causing blood pressure fall [3]. Several analytical methods, including spectrophotometric methods [17-22], spectrofluorometric [23], chromatographic methods [24, 25], titrimetric method [26] and Volta metric method [27] have been reported for its determination alone or in combination.

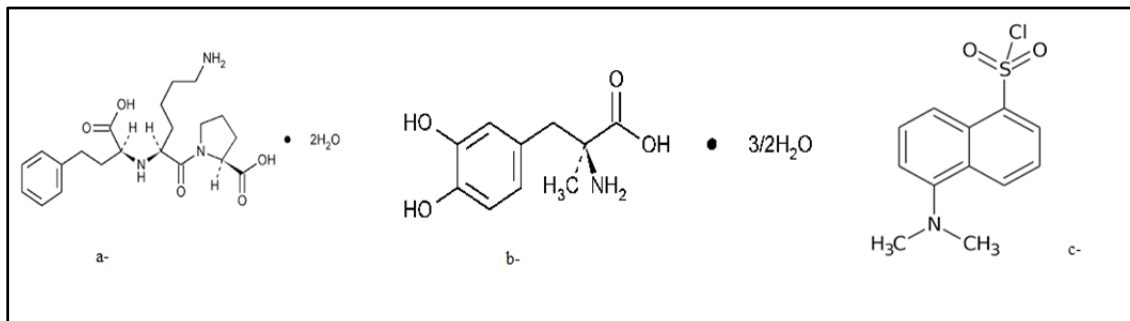


Figure 1: Chemical structure of [a-lisinopril], [b-methyldopa] and [c-dansyl-Cl].

2. Experimental

2.1. Apparatus

- Fluorescence measurements were carried out on a FP-6300 spectro-fluorimeter (Jasco, Japan) equipped with a 150 W xenon lamp and 1 cm quartz cells.
- Consort P400[®] digital pH-meter for pH adjustment.

2.2. Materials and reagents

All solvents and reagents used throughout the work were of analytical grade and double distilled water was used.

Lisinopril pure authentic sample (99%, purity) was kindly supplied by Ramedia, Egypt. **Sinopril**[®] tablets, labeled to contain 10 mg of lisinopril, batch no. b23205 (GNP, Egypt). **Methyl dopa** pure authentic sample (99.6%, purity) was kindly supplied by Eipico, Egypt. **Aldomet**[®] tablets, labeled to contain 250mg of Methyl dopa batch no. S420171 (KAHIRA, Egypt). **Dansyl chloride (DNS-Cl)** was purchased from Cornell Lab Company, solution of DNS-Cl was freshly prepared at 3.0 mg/ml in acetone. (Stable for one week). **Sodium hydroxide** was purchased from Research lab fine chemical industry, solution of NaOH was freshly prepared at 20 mg/ml in distilled water and pH was adjusted by 1M of (34%) HCl to 8.5.

2.3. General procedures

2.3.1. Preparation of stock standard drug solution: A stock standard solution of lisinopril (1mg/ml) was prepared by weighing accurately 0.1 gm of pure drug and dissolving in 100 ml distilled water. stock standard solution of methyl dopa (1mg/ml) was prepared by weighing accurately 0.1 gm of pure drug and dissolving in 100 ml methanol.

2.3.2. Preparation of working standard solution and construction of calibration curve: Aliquot containing from 1 to 25 µg/mL of (LIS) and (MD) was prepared by transferring certain increasing volume from each drug to a series of 10 ml volumetric flasks, followed by adding 0.3 ml of standard NaOH, mixed well, then 0.5 ml of standard (DNS-Cl) solution was added, mixed and left for 30 minutes for [LIS] and 35 minutes for [MD] in dark at 35 °C.

The flasks were then cooled and the volume was completed to 10 ml with methanol. The fluorescence intensity of the resulting solution was measured at 511 and 516 nm for (LIS) and (MD) respectively after excitation at 323 nm against reagent blank that had been treated similarly. The fluorescence intensity was plotted versus the final drug concentrations to get the calibration curve. The corresponding regression equation was computed.

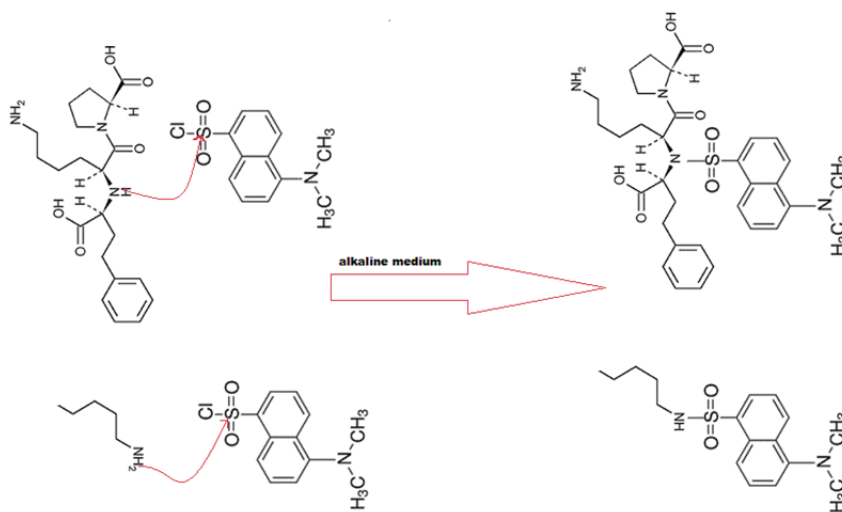
2.4. Application on pharmaceutical preparations

Ten tablets of Sinopril® and five tablets of Aldomet® were crushed, powdered and the average weight of one tablet was determined. Then specific weight from each powdered drug equivalent to 100 mg was dissolved in 100 ml distilled water for sinopril® and methanol for aldomet® then filtered through whatman filter paper to give a final stock concentration of 1mg/ml for each drug. Working solutions were prepared by serial dilution from stock solution. The procedures were then completed as previously mentioned using standard addition technique.

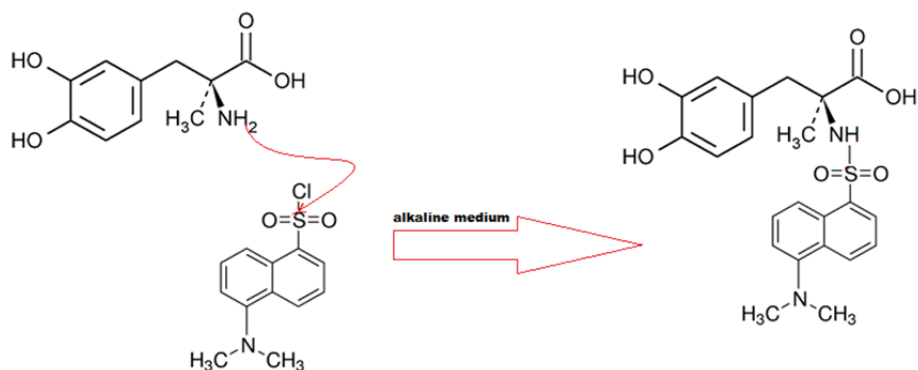
3. Results and Discussion

3.1. Fluorescence spectra

Both (LIS) and (MD) possess amino groups which have the ability to react with dansyl chloride in alkaline medium resulted in formation of a high fluorescent product, while the reagent blank exhibited a weak fluorescence intensity at the selected excitation and emission wavelengths under the optimized experimental conditions. Scheme 1 and 2) illustrates the suggested reaction pathway for both drugs with dansyl chloride. Figure 2 and 3 explain the Fluorescence spectra for both drugs.



Scheme 1: Suggested reaction pathway between Lisinopril and dansyl chloride.



Scheme 2: Suggested reaction pathway between methyl dopa and dansyl chloride.

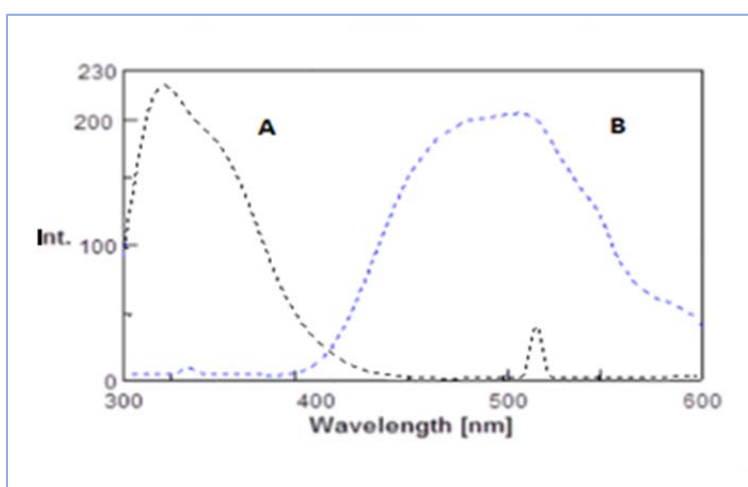


Figure 2: Excitation (A/black) and emission (B/blue) spectra of the LIS-DNS product ($\lambda_{ex} = 323$ nm and $\lambda_{em} = 511$ nm).

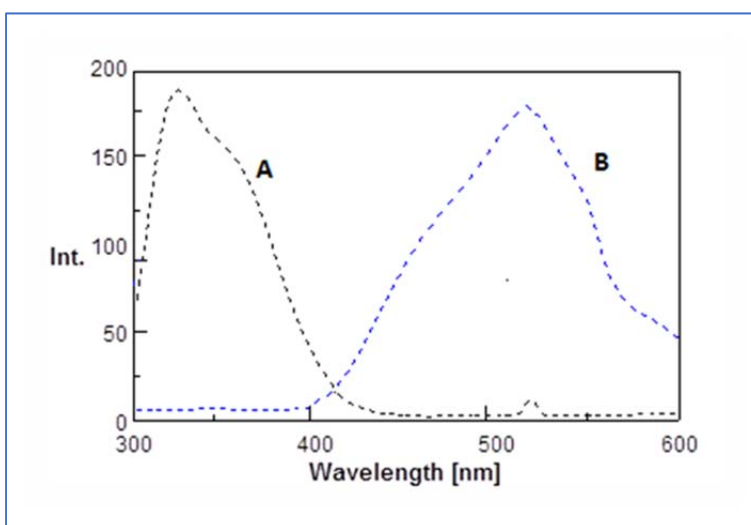


Figure 3: Excitation (A/black) and emission (B/blue) spectra of the MD-DNS product ($\lambda_{ex} = 323$ nm and $\lambda_{em} = 516$ nm).

3.2. Optimization of reaction conditions

Different experimental factors including pH, volume of buffer, amounts of reagent, temperature and reaction time were studied and optimized by changing each factor individually keeping the others constant.

3.2.1. Effect of pH: The influence of pH on the fluorescence intensity of the reaction product was examined using sodium hydroxide solution over the pH range from 6 to 11.0 since DNS-Cl reacts under alkaline conditions. The maximum fluorescence intensity was obtained when the reaction was carried out with NaOH solution of pH 8.5. Figure 4 illustrate the effect of pH.

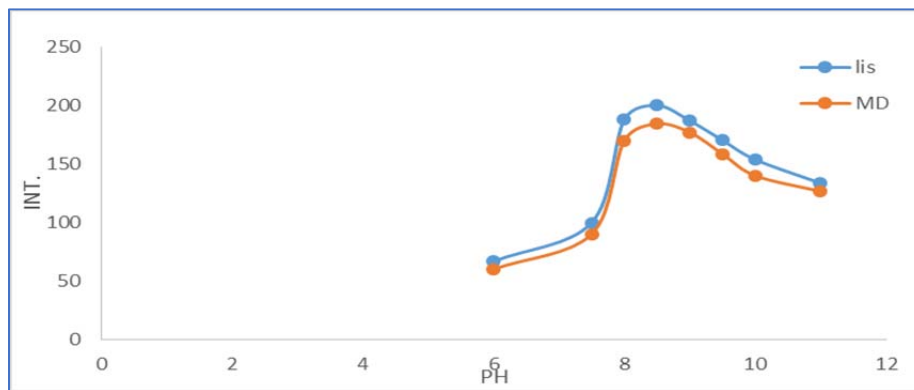


Figure 4: Effect of the pH on the development of the reaction product of (LIS) and (MD) (10 $\mu\text{g}/\text{mL}$) with DNS-Cl.

3.2.2. Effect of sodium hydroxide volume: The influence of volume of NaOH on the fluorescence intensity of the reaction product was examined using different volume over the range from 0.1 to 0.7 ml. Maximum fluorescence intensity was obtained when the reaction was carried out using 0.3 ml NaOH solution of pH 8.5 as shown in Figure 5.

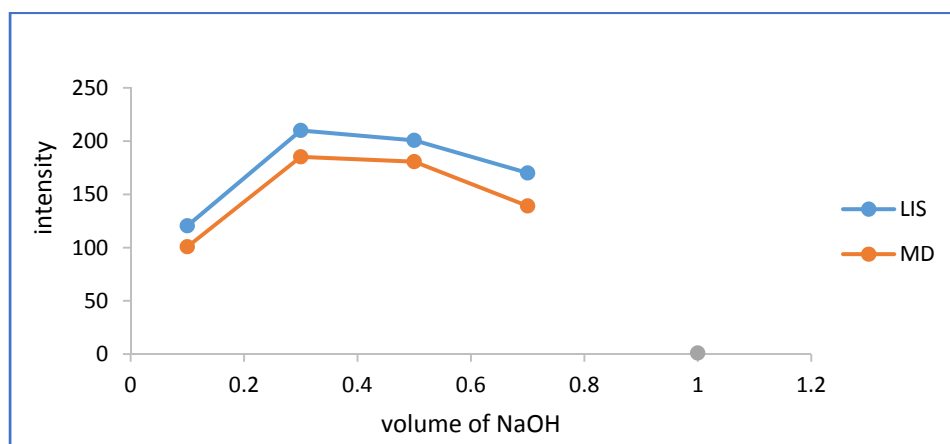


Figure 5: Effect of volume of NaOH solution on development of the reaction product of (LIS) and (MD) (10 $\mu\text{g}/\text{mL}$) with DNS-Cl.

3.2.3. Effect of dansyl chloride volume: The influence of the volume of dansyl chloride solution was examined by addition of different volumes of 3.0 mg/ml reagent in the range of 0.2 to 1 ml. A maximum fluorescence intensity was obtained when 0.5 ml of dansyl chloride solution was utilized as depicted in Figure 6.

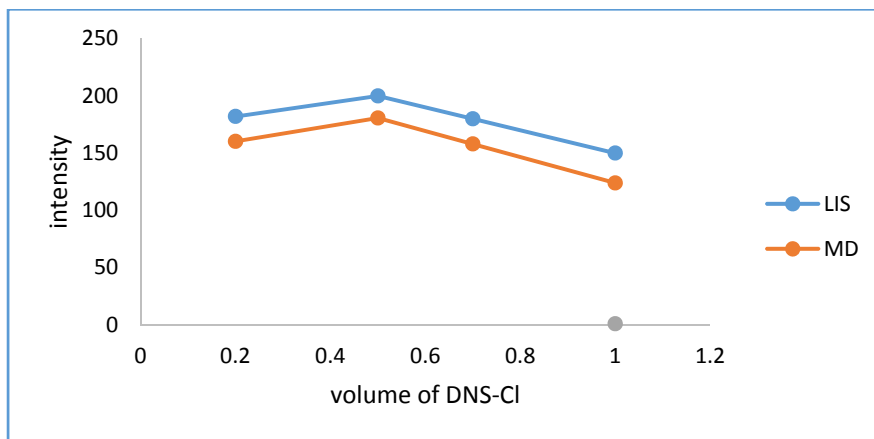


Figure 6: Effect of volume of DNS-Cl solution on development of the reaction product of (LIS) and (MD) (10 µg/mL).

3.2.4. Effect of time and temperature: In this study, the reaction between the two drugs and dansyl chloride was performed using pH 8.5 at different temperatures (25°C, 35°C, 40°C, and 50°C) for various time intervals (10, 20, 30, 35 and 40 min). As it is seen in Figure 7 and 8, the reaction was found to be completed after 30 and 35 min for (LIS) and (MD) respectively at 35°C.

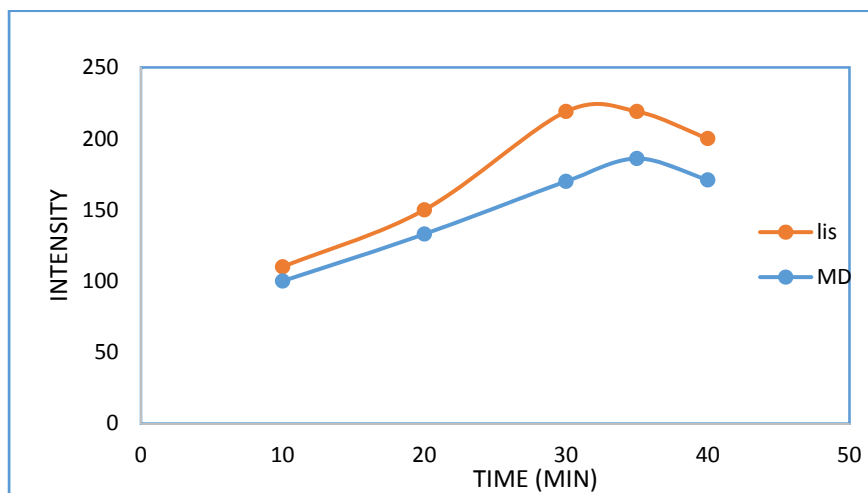


Figure 7: Effect of time on the reaction completion of (LIS) and (MD) (10 µg/mL) with DNS-Cl.

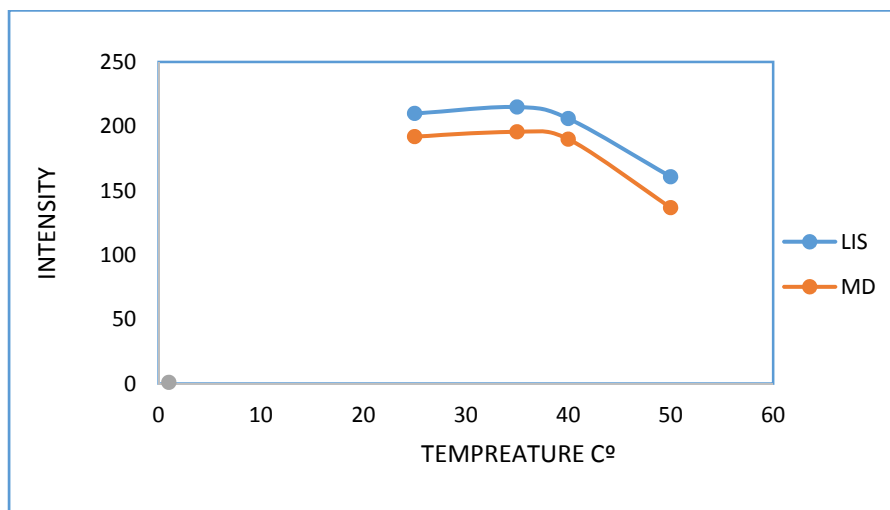


Figure 8: Effect of temperature on the reaction completion of (LIS) and (MD) (10 µg/mL) with DNS-Cl.

3.3. Stoichiometry of the reaction

The molar ratio of the reagent and the two drugs in the reaction was studied by using the continuous variation method (Job's method) [28]. The molar ratio was found to be 12 (drug: reagent) and 1:1 (drug: reagent) for Lisinopril and methyl- dopa respectively as seen in Figure 9.

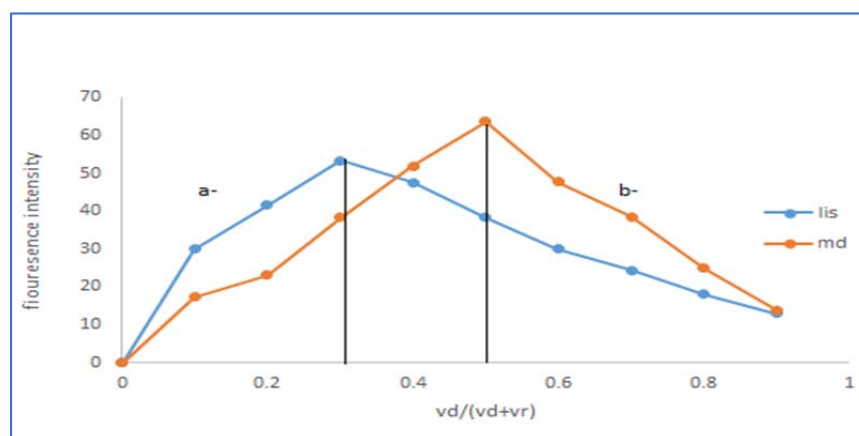


Figure 9: Continuous variation plots for the reaction between: (A) 2.26×10^{-4} M of DNS-Cl and 2.26×10^{-4} M of Lisinopril and (B) 4.2×10^{-4} M of DNS-Cl and 4.2×10^{-4} M of Methyl-dopa.

3.4. Method validation

The validity of the proposed method was tested regarding linearity, range, limits of detection, limits of quantification, accuracy, precision, robustness and specificity according to ICH recommendations [29].

3.4.1. Linearity: Under the optimum experimental conditions, standard calibration curves were constructed at five concentration levels by plotting the values of fluorescence intensity versus the final concentrations (µg/ml) as shown in Figure 10. The correlation coefficient was 0.9999 and 0.9997 for (LIS) and (MD) respectively, indicating good

linearity over the concentration range of 3.0 to 20.0 and 7.0 to 25.0 µg/ml for (LIS) and (MD) respectively. The intercept, slope, Correlation coefficient for the calibration data are summarized in Table 1. Calibration graph is described by the equation (Y= a+bX), (Where Y= florescence intensity, a= intercept, b= slope and X= concentration in µg.ml⁻¹).

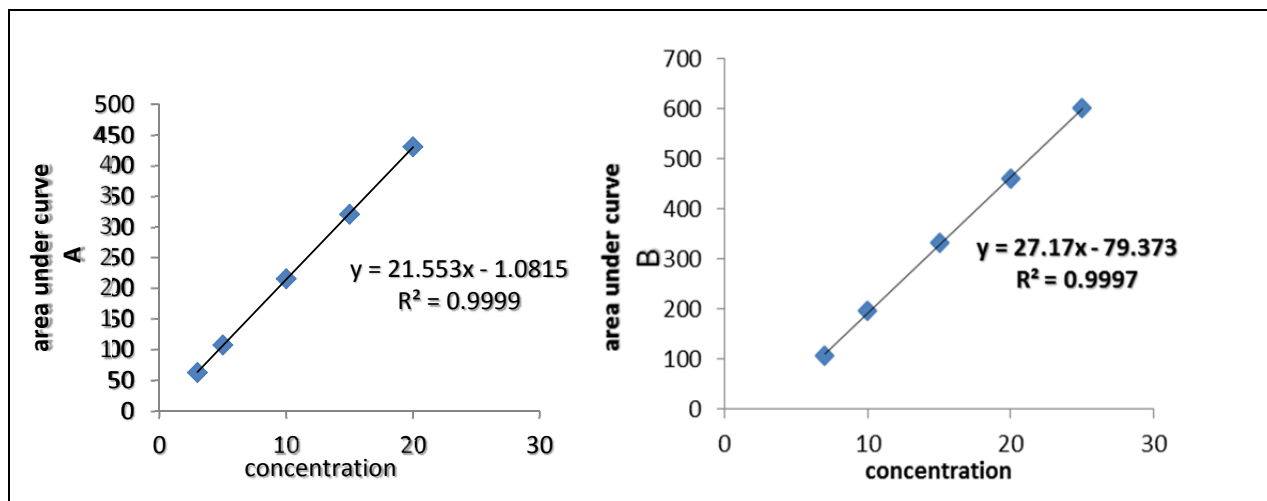


Figure 10: Calibration curves for the reaction between (DNS-CL) and (LIS) (A) and (MD) (B) respectively.

Parameters		Lisinopril	Methyl dopa
Excitation wave length, nm		323	323
Emission wave length, nm		511	516
pH		8.5	8.5
Volume of NaoH (ml)		0.3	0.3
(DNS-Cl) volume (ml)		0.5	0.5
Time for derivatization reaction		30	35
Temperature (°C)		35 ± 2°C	35 ± 2°C
Solvent		water	Methanol
Beer's law limits (µg.ml ⁻¹)		3-20	7-25
Regression equation*	Slope (b)	1.0815	79.373
	Intercept (a)	21.553	27.17
Correlation coefficient		0.9999	0.9997

Table 1: Statistical and analytical parameters of (LIS) and (MD) determination.

3.4.2. Limits of detection and limits of quantification: The limit of detection (LOD) was calculated according to the equation $[LOD = 3.3 S / K]$. The limit of quantification (LOQ) was calculated by the equation $[LOQ = 10 S/K]$ Where S is the standard deviation of the three replicate determination and K is the slope of calibration graph. The results are summarized in Table 2.

Parameters	Lisinopril			Methyl dopa		
	Conc. taken	Conc. found	Recovery %	Conc. taken	Conc. found	Recovery %
	$\mu\text{g/ml}$	$\mu\text{g/ml}$		$\mu\text{g/ml}$	$\mu\text{g/ml}$	
	3	2.959	98.64	7	6.8779	98.2559
	5	5.03788	100.757	10	10.124	101.241
	10	10.0506	100.506	15	15.0958	100.638
	15	14.922	99.482	20	19.8757	99.3785
	20	20.0288	100.144	25	25.0266	100.106
Mean*			99.9			99.92
N			5			5
SD			0.85			1.1575
RSD			0.85			1.1584
SE			0.427			0.5787
Variance			0.729			1.3399
LOD, μgml^{-1}			0.264			0.2097
LOQ, μgml^{-1}			0.88			0.699
Apparent Molar absorptivity**			9435577			4957090
L $\text{Mol}^{-1} \text{cm}^{-1}$						

*Mean of three different experiments; ** Calculated in the basis of molecular weight of the drug.

Table 2: Statistical data for the reaction of lisinopril and methyl dopa with dansyl chloride.

3.4.3. Accuracy and precision: Accuracy of the proposed methods was checked by performing recovery experiments through standard addition technique. The results are shown in Table 3 indicates good accuracy. The precision of the method was calculated in term of intermediate precision (intraday and inter-day). Three different concentrations five times of lisinopril and methyl dopa were analyzed during the same day (intra-day precision) and five consecutive days (inter-day precision). The standard analytical errors, relative standard deviations (RSD) and recoveries obtained by the proposed method were found to be acceptable. The results are summarized in Table 4.

	Sinopril® tablets				Aldomet® tablet			
	Take from Sinopril tablet (µg/ml)	Added from pure drug (µg/ml)	Conc. found (µg/ml)	Recovery* %	Taken from aldomet tablet (µg/ml)	Added from pure drug (µg/ml)	Conc. found (µg/ml)	Recovery* %
	3	0	2.936	98	7	0	6.879	98.28
	3	2	5.0237	100.474	7	3	10.1218	101.218
	3	7	10.07	100.735	7	8	15.095	100.639
	3	12	14.9	99.399	7	13	19.874	99.372
	3	17	20.056	100.281	7	18	25.027	100.108
	3	21	23.95	99.796	-	-	-	-
Mean*	-	-	-	99.76	-	-	-	99.923
N	-	-	-	6	-	-	-	5
S.D.	-	-	-	1.03595	-	-	-	1.14
R.S.D.	-	-	-	1.0384	-	-	-	1.14
V	-	-	-	1.07	-	-	-	1.3
S.E.	-	-	-	0.5179	-	-	-	0.57

*Mean of three different experiments.

Table 3: Application of standard addition technique for the determination of Sinopril® and Aldomet® tablets form through reaction with DNS-Cl.

Item	Concentration µg/ml	Intraday		Inter-day	
		Mean ±SD	RSD	Mean ±SD	RSD
Lisinopril	10 µg/ml	100.5 ± 0.15	0.15	100.46 ± 0.28	0.28
	15 µg/ml	99.655 ± 0.244	0.245	99.2 ± 1.23	1.24
	20 µg/ml	100.117 ± 0.1688	0.1686	100.108 ± 0.29	0.29
Methyldopa	10 µg/ml	101.156 ± 0.4	0.39	101.2 ± 0.63	0.62
	15 µg/ml	100.465 ± 0.197	0.196	100.6 ± 0.208	0.206
	20 µg/ml	99.19 ± 0.185	0.1867	99.349 ± 0.637	0.642

Table 4: Results of the intraday and inter-day precision for the determination of lisinopril and methyl dopa using DNS-Cl.

3.4.5. Robustness: Robustness was achieved by making small incremental change in volume of sodium hydroxide and volume of reagent. The effect of changes was studied on the percent recovery of drugs proved that all changes had a negligible influence on the results as seen in Table 5.

Robustness		
% of recovery \pm SD		
Item	Lisinopril	Methyl dopa
NaOH+0.05ml	99.9 \pm 0.814	99.9\pm1.65
NaOH-0.05 ml	99.99 \pm 0.716	99.9\pm 1.15
DNS-Cl+0.05ml	99.8 \pm 1.17	99.9\pm0.888
DNS-Cl-0.05ml	99.88 \pm 0.8	99.948\pm 0.7

Table 5: Results of the robustness for the determination of lisinopril and methyl dopa using DNS-Cl.

3.4.6. Analysis of pharmaceutical preparations: The proposed methods were applied to the analysis of the drug in dosage forms and the results were statistically compared with reported reference methods by calculating Student's *t*- and F-values. The evaluated *t*- and F-values were less than the tabulated values at the 95% confidence level. Results listed in Table 6 showing that there is no statistical significance difference between the proposed and reference methods.

Statistics	Sinopril [®] tablet		Aldomet [®] tablet	
	Reported reference method number [133]	Proposed method	Reported reference method number [153]	Proposed method
Mean recovery* \pm SD	100.33 \pm 0.7	99.76 \pm 1.0359	100.1 \pm 1.2	99.9 \pm 1.14
N	6	6	5	5
Variance	0.49	1.07	1.44	1.3
t-test**	-	1.1 (2.23) ^a	-	0.27(2.3) ^a
F-ratio**	-	2.18 (5.05) ^b	-	1.1 (6.39) ^b

* Average of three experiments; ^a and ^b are Theoretical Student *t*-values and F- ratio at $p=0.05$.

Table 6: Statistical data for the determination of pharmaceutical tablets Sinopril[®] and Aldomet[®] through the proposed methods using DNS-Cl compared with the reference methods.

4. Conclusion

The proposed method was simple, fast, selective, sensitive, specific, reproducible and not very expensive. Validation experiments proved that results were linear over the mentioned working range. No interference was observed from the described drugs and their common excipients. Also there was no need for extraction procedure.

5. Acknowledgment

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