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Research Article

Spectrophotometric Determination of Dopamine in Bulk and Dosage Forms Using 2,4-Dinitrophenylhydrazine

2,4-Dintrofenilhidrazin Kullanilarak Dopaminin Ham Ürün Ve Dozaj Formlarinda Spektrofotometrik Tayini

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Abstract:
Objectives: Dopamine hydrochloride (DA) is a sympathomimetic agent, used therapeutically for the correction of hemodynamic disorders associated with shock episodes. Although several analytical methods were described, spectroscopic assay of DA after chemical derivatization with 2,4-dinitrophenylhydrazine (DNP) is still unexamined. Therefore, the optimization of the reaction parameters and validation of developed method were required.
Materials and Methods: The method is based on coupling of DA as phenolic compound with diazonium salt to produce an intensely colored azo-derivative. DNP was oxidized with potassium periodate (PPI) to produce a diazonium salt which coupled with DA in basic media. The experimental parameters were studied and optimized. The developed method was validated according to International Conference on Harmonisation (ICH) guidelines and was applied to dosage forms. The results were compared with data of a reference method.
Results: The method was linear in a concentration range between 5 and 50 µg/mL. The regression line equation was $Y = 0.042 \pm 0.0003X + 0.0672 \pm 0.0015$ with a regression coefficient of 0.9944 (n=5). The limit of detection (LOD) and limit of quantification (LOQ) were 0.32 and 0.97 µg/mL, respectively. The precision was satisfactory; the percentage relative standard deviation (RSD) had not exceeded 2%. The average values of recovery study were found to be in the range 98.90-100.40 ± 0.31–1.21%. The developed method was applied successfully for determination of DA in injection and infusion fluid.
Conclusion: The method is accurate, sensitive and practical for DA analysis in quality control laboratories.
Keywords: Dopamine hydrochloride, 2,4-Dinitrophenylhydrazine, Spectrophotometric, Validation.

ÖZ
Amaç: Dopaminhidroklorür (DA), şok vakarıyla ilişkili hemodinamik bozuklukların düzeltilmesinde terapötik olarak kullanılan bir sempatomimetik ajandır. Çok sayıda analitik yöntem tarif edilmesine rağmen, 2,4-dinitrofenilhidrazin (DNP) ile kimyasal türevlendirme sonrası DA'nınspektroskopik analizi henüz incelenmemiştir. Bu nedenle, reaksiyon parametrelerinin optimizasyonu ve geliştirilen testin geçerliliği gereklidir.
Gereç ve Yöntemler: DA'nınfenolik bileşik olarak bazik ortamda diazonyum tuzu ile birleştirilmesine dayanan kimyasal türevlendirme, UV-spektroskopik ölçümlerinden önce kırmızı bölgeye kaydırılmış azo-türevini türetmek için uygulanmıştır. Diazonyum tuzu, DNP'nin potasyum periyodat (PPI) ile oksidasyonu ile hazırlandı. Reaksiyon parametreleri

değerlendirilerek optimize edildi. Geliştirilen analiz ICH kılavuzlarına göre doğrulandı ve dozaj formlarına uygulandı. Sonuçlar bir referans yönteminin verileriyle karşılaştırıldı.
Bulgular: Yöntemin doğrusallıkaralığı 5 ila 50 ug / mL arasındadır. Regresyon çizgisi denklemi Y = 0.042 ± 0.0003X+0.0672 ± 0.0015, regresyon katsayısı ise 0.9944 (n = 5) olarak saptandı. Teşhis limiti (LOD) ve hesaplama limiti (LOQ) sırasıyla 0,32 ve 0,97 µg /mL olarak tespit edildi. Hassasiyet düzeyi yeterli seviyede olup; yüzde bağıl standart sapma (RSD) % 2'nin altında kaldı. Geri kazanım çalışmasının ortalama değerleri % 98.90-100.40 ± 0.31-1.21 arasında bulundu. Geliştirilen yöntem enjeksiyon ve infüzyon sıvısında DA tayini için başarıyla uygulanmıştır.
Sonuç: Yöntem, kalite kontrol laboratuvarlarında DA analizi için doğru, hassas ve pratiktir.
Anahtar Kelimeler: Dopaminhidroklorür, 2,4-Dinitrofenilhidrazin, Spektrofotometrik, Validasyon.
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INTRODUCTION:

Dopamine hydrochloride (DA) is chemically named 1,2-benzenediol,4-(2-amonoethyl) hydrochloride. Dopamine is an endogenous catecholamine, which is a sympathomimetic agent with prominent dopaminergic and β_1-adrenergic effects at low to moderate doses and α-adrenergic effects at high doses. It is used for the correction of hemodynamic disorders associated with shock episodes.[1,2]
Literature survey of DA revealed several methods for its' determination in injection. Spectrophotometric methods using bromanil, 2,6-dichloroquinone-4-chloroimide (DCQ), 3-amiopyridine, chloramine T and various oxidative coupling based methods were published.[3-10]. In addition, different High Performance Liquid Chromatography (HPLC)[11-15], flow injection[16,17], fluorimetric[18] , capillary electrophoresis[19], chemiluminescence[20] and electrochemical[21-26] methods were reported.
Spectrophotometry is considered as the most practical analytical procedure in quality control laboratories, since it does not need costly instrumentation and toxic solvents as chromatography. 2,4-Dinitrophenylhydrazine (DNP) is a derivatizing agent, which was used in analysis of many drugs.[27-31] Chemical derivatization prior to spectroscopic analysis enhances both sensitivity and selectivity.[32]
The current study was performed - in continuation to our interest for developing and validation of simple, sensitive, and rapid spectrophotometric methods for analysis of drugs[33,34] - to determine DA depending on a derivatization reaction with DNP in pharmaceuticals.

MATERIALS AND METHODS:

Instruments:
Spectrophotometer: SHIMADZU UV-1601 with UV-Pro software(Shimadzu, Japan) and Lambda 25 with V5 ES software (PerkinElmer, USA) and 1 cm quartz cells(Innovative Lab Supply, USA) were used.
Materials:
All chemicals used were of analytical grade. Dopamine hydrochloride standard was purchased from (Merck, Germany). Dopamine dosage forms were ampoules for infusion (200 mg/5mL) and

dopamine hydrochloride with 5% dextrose infusion fluid (800 µg/mL DA) obtained from a local hospital pharmacy (Gaza, Palestine).

Preparation of reagents:

2,4-Dinitrophenylhydrazine (DNP,0.005 M) reagent: 0.10 g of DNP were accurately weighed and transferred into 100 mL volumetric flask, dissolved in 2.5 mL concentrated sulfuric acid and completed up the volume with distilled water. The solution was freshly prepared and protected from light during the use because it is light sensitive.

Potassium periodate (PPI, 0.0065 M) reagent: 0.15 g of PPI were accurately weighed and transferred into a 100 mL volumetric flask, dissolved and completed the volume with distilled water.

Sodium hydroxide (NaOH,10 M): 40.00 g of NaOH were accurately weighed and transferred into a volumetric flask, dissolved and completed the volume with distilled water to 100 mL.

Standard stock solution:

It was prepared by dissolving 0.02 g of dopamine hydrochloride standard in 100 mL distilled water (200 µg/mL). Working solutions were prepared by diluting the stock solution. The stock solution was freshly prepared during the use.

General procedure:

An aliquot of standard stock solution was transferred into a 10 mL volumetric flask followed by 1.0 mL DNP, 1.0 mL PPI, and 0.5 mL NaOH reagents. The mixture was mixed well and diluted to 10 mL with distilled water at room temperature. The absorbance was measured at λ_{max} 560 nm against blank.

Determination of stoichiometric ratio (Job's method):

Job's method of continuous variation was employed.[35] Equimolar solutions ($3*10^{-3}$ M) aqueous solution of DA and DNP were prepared. Series of 1.0 mL portions of DA and DNP were made up compromising different complementary volumes (0.0:1.0, 0.1: 0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.7:0.3, 0.8:0.2, 0.9:0.1, 1.0:0.0) in 10 mL volumetric flasks, respectively. The process followed general procedure. Absorbance was plotted against DNP molar fraction.

Optimization of reaction conditions:

Different reaction parameters were studied. They included concentration and volume of DNP, PPI, and NaOH, temperature, reaction time, order of addition and stability of developed chromogen. The study was carried out by altering one factor and keeping the others constant.

Method validation:

Validation parameters were carried out according to International Conference on Harmonisation ICH guidelines.[36]

Assay of pharmaceutical formulations:

The content of three ampoules for DA was mixed and an accurately measured volume equivalent to 0.020 g DA was transferred in 100 mL volumetric flask. Using distilled water to bring the volume up to 100 mL.

For DA and 5% dextrose infusion fluid, the content of three bottles was mixed and an accurately volume equivalent to 0.02 g was transferred into 100 mL volumetric flask and diluted with water. It was further diluted to get a concentration of working solutions. Analysis was performed as described in general procedure.

RESULTS:

In order to enhance sensitivity a derivatization reaction of DA using DNP was performed. A red shifted DA-derivative was produced, which showed an absorption maximum (λ_{max}) at 560 nm (Figure 1). The reaction parameters were optimized (Table 1). The reaction was completed immediately at room temperature. Heating is not advantageous due to intermediate –diazonium-

instability. To DA solution DNP was added followed by PPI and NaOH (Table 2). DNP is oxidized by PPI to form diazonium ion, and the pH was still acidic. Once diazonium formed it attacked electron rich phenolic DA. The last step requires basic media. Addition of PPI to DA (Table 2) was unsuitable since it can lead to oxidation of catechol moiety of DA. When NaOH was added to DA the absorption was decreased. This can be explained by phenoxide formation and inappropriate media for diazonium salt formation[37-39] The best result was achieved when 1mL of both DNP and PPI reagents and 0.5 mL NaOH were added to DA in the mentioned order. After dilution DA-derivative was stable for at least 15 minutes (Figure 2), which allowed processing of samples and their comfortable measurements. Stoichiometric ratio of DA-derivatization reaction was studied by Job's method. The molar ratio was 1:1 for DA and DNP (Figure 3). Accordingly, a proposed mechanism of reaction is illustrated in figure 4 depending on result of molar ratio and mechanism of azo-formation.

Method validation:

Linearity and sensitivity:

For evaluation of linearity DA was determined at optimized conditions for five concentrations. The calibration curve was $Y = 0.042 \pm 0.0003X+0.0672 \pm 0.0015$, ($r=0.9944$, $n=5$), where Y is the absorbance at 560 nm and X is the concentration of DA in (μg/mL). The linear range was 5-50 μg/mL. The molar absorptivity (ε) was 7.9×10^4 L/mol.cm. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as $3.3\sigma/S$, and $10\sigma/S$, respectively[36], where σ is residual standard deviation of regression line and S is slope of the regression line. LOD and LOQ were 0.32 and 0.97 (μg/mL), respectively.

Accuracy, precision, and specificity:

The accuracy was evaluated by the recovery studies for added standard concentrations to a pre-analyzed product at low, intermediate and high concentrations. The recovery values were 99.5-101.9 ± 0.21-1.12% (Table 3), indicating the accuracy of the method. Intra-day precision was assessed at three different concentrations by analyzing five replicates per concentration on the same day. Inter-day precision was determined by analyzing samples for 6 consecutive days within a week (Table 4). The percentage of relative standard deviation (RSD) did not exceed 2%, proving the high precision of the method. Before proceeding with the analysis of DA in its dosage forms, interference liabilities were carried out to examine the effect of excipients that might be added during formulation. Samples were prepared by mixing 10 and 40 mg DA with excipients like sodium bisulfate (0.05 g), and dextrose (5.0 g). These laboratory prepared samples were analyzed by the developed method. The recovery values were 98.9-100.4± 0.31-1.21% (Table 5). These data confirmed the absence of interference from excipients with DA determination by the developed method.

Robustness and ruggedness:

Robustness was evaluated by studying the influence of small variation in the method variables on its analytical performance. One parameter was changed whereas the others were kept unchanged and the recovery values were calculated each time. The recovery values were 98.6–101.1 ± 0.31–1.12 (Table 6). This indicated the reliability of the method. Regarding ruggedness, lab-to-lab variations were examined by performing DA analysis using the same operational conditions but using two different instrumentations. Results obtained were reproducible, as RSD did not exceed 1.43% (Table 7).

Application of the method:

DA-Pharmaceutical dosage forms (Ampoule, infusion fluid) were analyzed successfully by the developed method. Results comply with the USP 29 specifications of DA content in injection (95-105%).[40] By comparing the result with reference data[5] by statistical analysis with respect to

the accuracy by t-test, there was no significant difference at 95% confidence level. This confirms similar accuracy in the determination of DA by both methods (Table 8).

DISCUSSION:

Dopamine contains a catechol group, which can be coupled with a diazonium cation in basic solution to produce a red shifted azo-derivative. A simple one step procedure was achieved for spectrophotometric analysis of DA after derivatization with DNP. The reaction was completed immediately at room temperature, which is advantageous in comparison to other spectroscopic DA assays. Heating at 30, 40 and 75 °C and adjustment of pH using a buffer were required for 4-aminoantipyrine[10], bromanil[6], and 2-hydroxynaphthaldehyde[11] based spectroscopic analysis of DA, respectively. A derivatization reaction was described for spectroscopic analysis of DA based on the formation of intensely colored Prussian blue required 35 minutes for completetion.[9] In addition, the developed method exhibited enhanced sensitivity (ε 7.9×10^4 L/mol.cm). The recorded molar absorptivities (ε) for spectrophotometric DA determination were $3.475x10^3$, 6.47×10^3 and 7.4×10^3.[4,6] A HPLC assay for DA analysis showed a comparable range 12-40 (µg/mL) with the developed method.[12] Chromatographic methods require highly sophisticated instruments and expensive solvents.

Validation studies were in good agreement with ICH guidelines. The method was accurate, and precise. Results of interference liabilities proved specificity of the developed method, thus the method can be applied for DA analysis in its dosage forms. Statistical analysis showed the developed method is comparable with a reference method[5] for analysis of DA.

CONCLUSION:

The developed method has the advantages of being simple, rapid, sensitive, accurate low cost, and do not require any pretreatment of the drug. The developed method was applied successfully for analysis of DA in dosage forms without interference from excipients. Therefore, the developed method can be suitable for routine analysis of DA in quality control laboratories.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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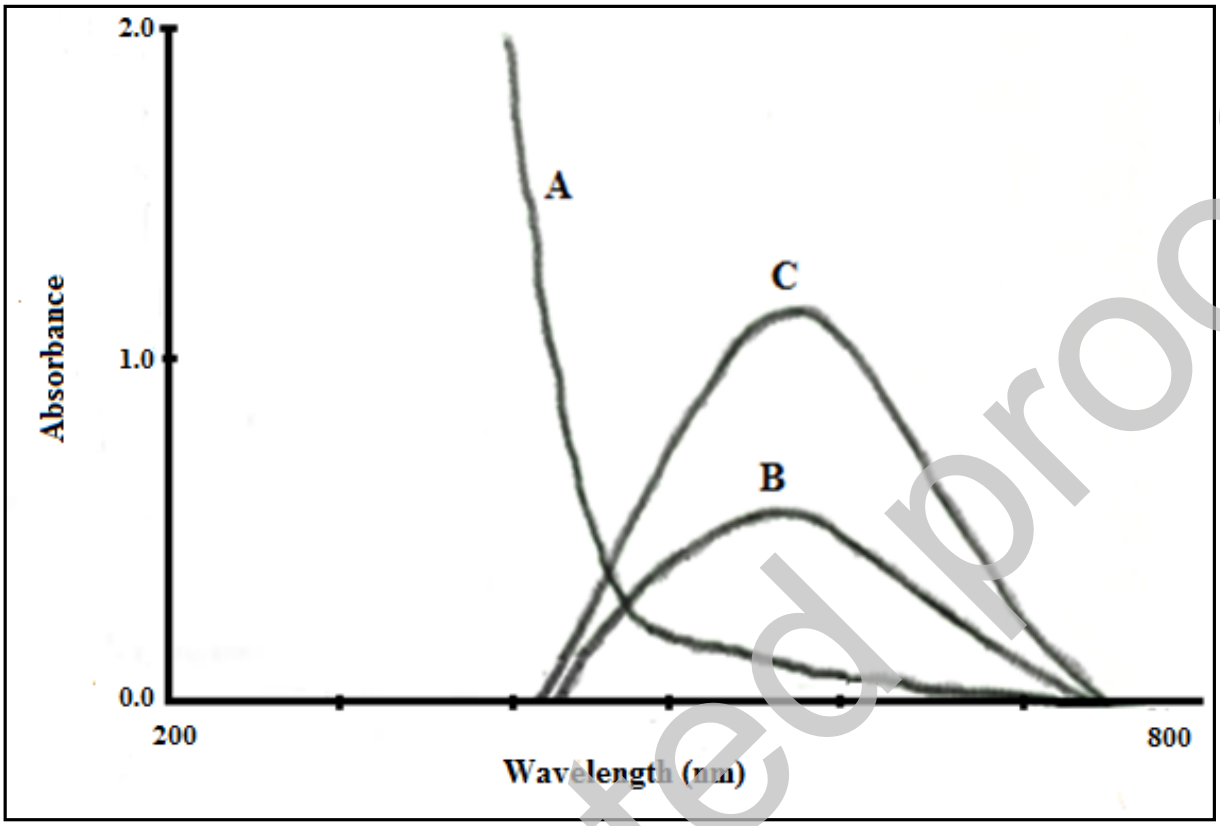

Figure 1: Absorption spectra. A: Blank spectrum against water; B and C: Derivatization products against blank (DA: 10 and 30 µg/ml), respectively.

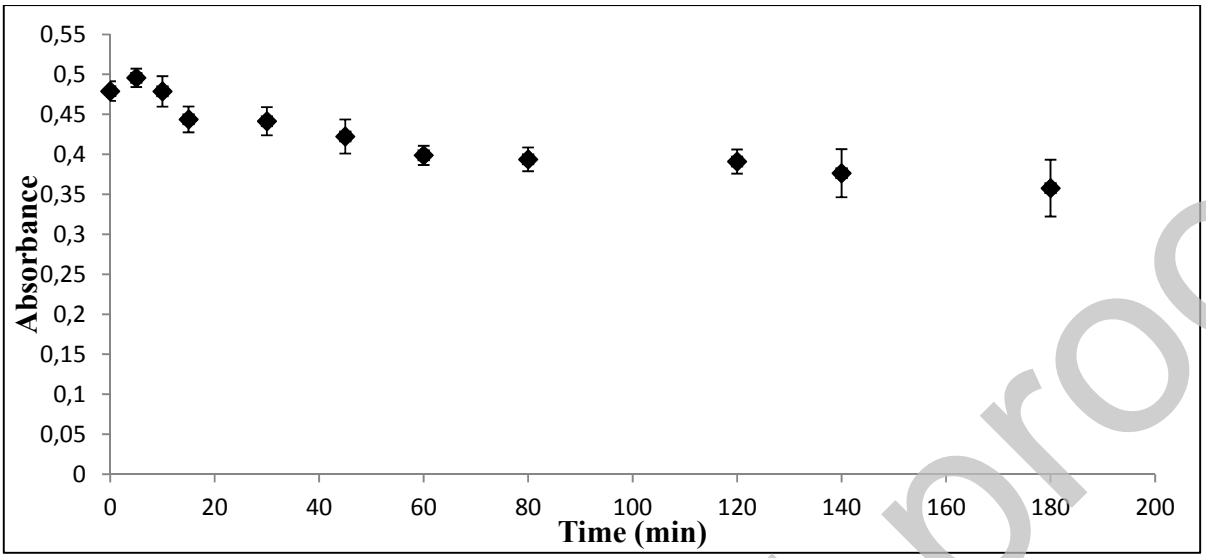

Figure. 2: Stability of chromogen resulting from the reaction of DA with DNP. DA (10 µg/mL), absorbance is average of three determinations. Error bars represent standard deviation.

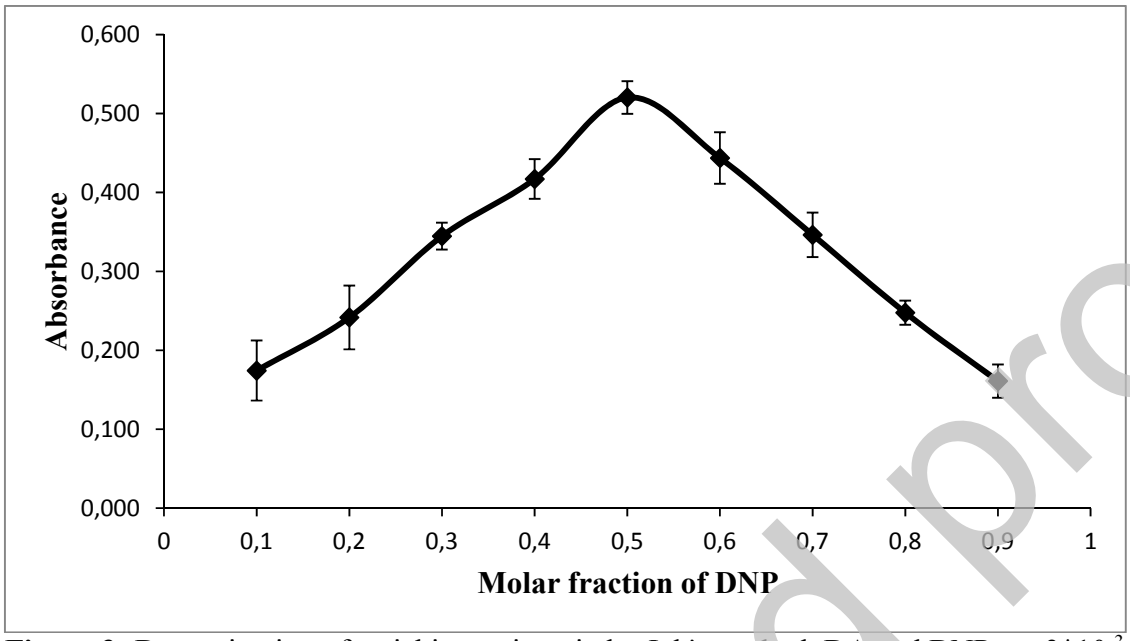

Figure 3: Determination of stoichiometric ratio by Job's method. DA and DNP are $3*10^{-3}$ M, absorbance is average of three determinations. Error bars represent standard deviation.

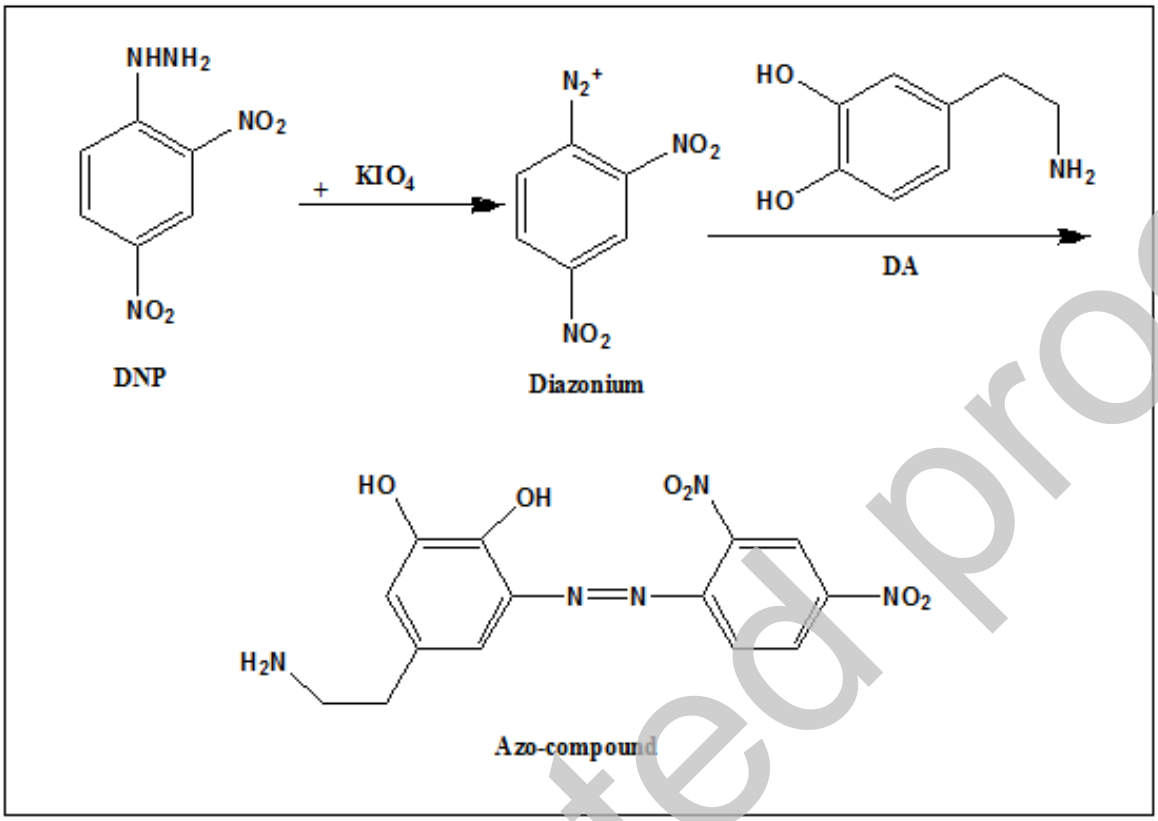

Figure 4: Suggested reaction of DA-derivatization reaction**.**

Table 1: Summary of optimum conditions for DA-derivatization reaction.

Variable	Studied range[a]	Optimum
DNP concentration	0.0015-0.010M	0.005 M
Volume of DNP (0.005 M)	0.5-2.5 mL	1.0 mL
PPI concentration	0.0017-0.011M	0.0065 M
Volume of PPI (0.0065 M)	0.5-2.5 mL	1.0 mL
NaOH concentration	5-10 M	10 M
Volume of NaOH (10 M)	0.2-2.0 mL	0.5 mL
Temperature	25-60 °C	25 °C
Time	0-60 min	0 min
Order of addition	Different	[b]

[a]: DA 10 µg/mL, DNP, PPI, and NaOH were mixed according to tested factor, H_2O diluting solvent, absorbance at 560 nm, [b]: For best order of addition see table 2.

Table 2: Effect of order of addition on DA analysis.

Sample	First	Second	Third	Fourth	Absorbance[a] (Mean ± SD)
1	DA	DNP	PPI	NaOH	0.473 ± 0.005
2	DA	PPI	DNP	NaOH	0.451 ± 0.013
3	DA	NaOH	PPI	DNP	0.442 ± 0.001
4	DA	NaOH	DNP	PPI	0.447 ± 0.001
5	DNP	PPI	DA	NaOH	0.450 ± 0.010
6	DNP	PPI	NaOH	DA	0.438 ± 0.009

[a]: Values were mean of three determinations; DA 10 µg/mL, DNP (0.005 M, 1.0 mL), PPI (0.0065 M, 1.0 mL), NaOH (10 M, 0.5mL), H_2O diluting solvent, at room temperature, absorbance at 560 nm.

Table 3: Recovery studies for determination of DA by the developed method.

Pre-analyzed product[a] (µg/mL)	**Added DA (µg/mL)[b]**	**Recovery% (Mean ± SD)[c]**
10		
	5	101.2 ± 0.83
	10	99.5 ± 1.01
	15	100.8 ± 0.98
15		
	7.5	100.4 ± 1.12
	15	101.8 ± 0.21
	22.5	100.4 ± 0.57
20		
	10	101.9 ± 0.30
	20	100.5 ± 0.73
	30	100.2 ± 0.81

[a]: DA ampoule labeled to contain 200 mg/5 mL, found 198.6 ± 0.1 mg/5 mL by the developed method, appropriate dilution was done, [b]: Standard DA was added to a pre-analyzed product at 50%, 100%, and 150%, [c]: Values were mean of three determinations.

Table 4: Evaluation of intra- and inter-day precision.

DA concentration (µg/mL)	Intra-day, (n=5)	Inter-day, (n=6)
	RSD%	RSD%
5	0.99	1.5
20	1.14	0.65
40	0.64	0.42

RSD: Relative Standard Deviation

Table 5: Interferences liabilities from excipients.

Excipients	**DA concentration (µg/mL)**	**Recovery% (Mean ± SD)[a]**
Sodium bisulfite		
	10	99.21 ± 0.31
	40	100.4 ± 0.73
Dextrose		
	10	98.9 ± 1.21
	40	99.6 ± 0.58

[a]: Values were mean of three determinations.

Table 6: Robustness of the method.

Parameter	Variation	Recovery% (Mean ± SD)[a]
DNP concentration		
	0.004 M	99.6 ± 0.41
	0.006 M	98.9 ± 0.64
Volume of 0.005 M DNP		
	0.8 mL	101.1±0.93
	1.2 mL	100.5±0.76
PPI concentration		
	0.0057 M	98.6 ± 1.12
	0.0074 M	99.3 ± 0.31
Volume of 0.0065 M PPI		
	0.8 mL	100.2 ± 0.86
	1.2 mL	99.1 ± 0.37
NaOH concentration		
	9.8 M	98.7 ± 0.54
	10.2 M	99.2 ± 0.62
Volume of 10 M NaOH		
	0.4 mL	101.5 ± 1.5
	0.6 mL	99.3 ± 1.5
Temperature		
	23 °C	99.25 ± 0.14
	27 °C	100.55 ± 0.39

[a]: Values were mean of three determinations; The concentration of DA was 20 µg/mL.

Table 7: Ruggedness of the method.

DA concentration (µg/mL)[a]	Shimaduz UV-1601		PerkinElmer Lambda 25	
	Recovery% (Mean ± SD)	**RSD%**	**Recovery% (Mean ± SD)**	**RSD%**
5	98.3 ± 1.41	1.43	98.6 ± 1.22	1.24
20	100.7 ± 0.47	0.47	98.8 ± 0.58	0.59
40	98.6 ± 0.43	0.44	99.7 ± 0.29	0.29

[a]: Three determinations per concentration, RSD: Relative Standard Deviation.

Table 8: Determination of DA in dosage forms by developed method and comparison with reference data.

Dosage form[a]	**Recovery% (Mean ± SD)**[b]	
	DNP method	**Reference data**[5]
Ampoule	99.32 ± 0.51 (t = 1.6567, p-value = 0.1362)	98.56 ± 0.89
DA and dextrose infusion solution	95.81±0.87	-

[a]: labeled to contain 200 mg/5 mL DA per ampoule or 0.8 mg/mL DA and 5% dextrose infusion solution; [b]: Values are mean of five determinations, p-value > 0.05 insignificant difference.