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Analytical uses of charge-transfer complexes: determination of dosage forms of desloratadine

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Abstract

The charge-transfer interaction of desloratadine (DES) with 2,4-dichloro-6-nitrophenol (DCNP), 2,4-dinitrophenol (DNP) and picric acid (PA) reagents was used for the spectrophotometric determination of desloratadine in acetone-methanol (90:10 v/v) medium in case of DCNP and (80:20 v/v) medium in case of DNP and in chloroform in case of PA. The colored products are quantified spectrophotometrically at 402, 426, and 352 nm for DCNP, DNP and PA reagents, respectively. Optimization of the different experimental conditions is described. The calibration graphs are linear in the concentration range from 3.11-93.35, 3.11-62.17 and 3.11-43.44 μg $mL^{-1}$of DES using DCNP, DNP and PA reagents, respectively. Different analytical parameters, namely molar absorptivity (ε), standard deviation, relative standard deviation, correlation coefficient, limit of detection and quantification are calculated. The proposed methods were applied successfully to the determination of DES, either in pure and dosage forms, with good accuracy and precision. Mean recovery is found to be 100.2 ± 0.025, 99.9 ± 0.037 and 100.5 ± 0.09 for (Aerius) tablet and 99.85 ± 0.055, 99.95 ± 0.058 and 99.98 ± 0.047 for Desa tablet using DCNP, DNP and PA reagents, respectively. For more accurate results, Ringbom optimum concentration ranges were 9.32-62.16, 3.11-49.73 and 3.11-31.08 μg mL^{-1} DES drug and the correlation coefficients are found to be 0.9996, 0.9998 and 0.9999 using DCNP, DNP and PA reagents, respectively. The performance of the proposed methods was judged by calculating t- and F-values.The results obtained by the proposed methods are in good agreement with those obtained by the reported method as indicated by the percent recovery values. No interference was observed from common excipients present in pharmaceutical formulations.

Keywords: spectrophotometry, charge transfer complexes, desloratadine, drug formulation

Introduction

Desloratadine (DES) is indicated for the relief of the nasal and non-nasal symptoms of seasonal allergic rhinitis in patients 2 years of age and older. Its chemical designation is 8-chloro-6,11-dihydro-11-(4-piperdinylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine (Fig. 1). In the literature there were limited LC methods have been reported for determination of DES in pharmaceutical preparations. The assay method (Qi et al. 2005) reported describes the separation of degradation impurities from DES formed through forced degradation studies, but it was out of scope because it did not separate and determine the impurities. Reversed phase-

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high performance liquid chromatography (RP-HPLC) method has been reported for quantification of DES in pharmaceutical forms (Alam Razib el al. 2006) spectophotometric, spectrofluorometric and HPLC methods have been reported for determination DES in dosage forms and in human plasma (El-Enany et al. 2007) Liquid chromatographic (LC) method has been reported for simultaneous determination of loratadine and DES in pharmaceutical preparations using micro-emulsion as eluent c but forced degradation study and impurity details were not reported in these articles (Alam Razib el al. 2006, El-Enany et al. 2007, El-Enany et al. 2007). Ultra-performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about nine fold decreases in analysis time as compared to the conventional HPLC system using 5μm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3μm particle size analytical columns without compromise on overall separation (Mazzeo et al. 2005, Nguyen et al. 2006, Villiers et al. 2006, Wren and Tchelitcheff 2006, Russo et al. 2008). Gradient stability-indicating RP-UPLC method was developed and validated for the quantitative determination of desloratadine and its five impurities in pharmaceutical dosage forms (Raoa et al. 2010). π–Acceptors such as 2,4-dichloro-6-nitrophenol (DCNP), 2,4-dinitrophenol (DNP) and picric acid (PA) are known to yield charge-transfer complexes (Preimer and Speiser 1983) and radical ions with a variety of electron donors such as amines. As DES has no functional group that enables absorption in the visible region, we decided to analyze the drug through derivatization reactions. In this approach, three labeling agents, namely, 2,4-dichloro-6-nitrophenol (DCNP), 2,4-dinitrophenol (DNP) and picric acid (PA) have been used in derivatization reactions, based on their reaction with the free secondary amino group in the piperidine ring of DES. The proposed methods are highly specific for DES in the presence of the parent drug (loratadine) which contains a single basic center pyridine nitrogen atom. The main task of this study is to find fast, cheap, accurate and sensitive spectrophotometric method for the determination of DES, in raw material and in some commercial pharmaceutical preparations.

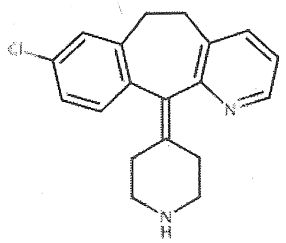


Figure 1. Structure of desloratadine

Materials and Methods

Apparatus

The spectrophotometric measurements were carried out using Shimadzu 1601 double beam UV-Vis spectrophotometer with quartz cells of 1 cm optical length incorporated with a pc computer loaded with Shimadzu UVPC software.

Materials

All chemicals and reagents used in this investigation were of analytical grade except otherwise specified. Desloratadine reference standard and bulk powder was supplied from Delta Pharma, Egypt. Desloratadine bulk powder was used as working standard and its purity was found to be 99.78% according to the manufacturer's method. The commercial formulations used included Aerius tablets (5mg/tablet, Schering-Plough, Belgium), Desa tablets (5mg/tablet, Delta Pharma, Egypt). Reagents included 2,4-dichloro-6-nitrophenol, 2,4-dinitrophenol and picric acid were purchased from Sigma-Aldrich, USA. Organic solvents included ethanol, methanol, acetone, chloroform, 1,4-dioxane, 1,2-dichloroethane, acetonitrile dimethylformamide and carbontetrachloride were supplied from El-Nasr Company, Egypt.

2,4-dichloro-6-nitrophenol (DCNP), 2,4-dinitrophenol (DNP) and picric acid (PA) solutions

10^{-3} M solutions of DCNP and DNP were prepared by dissolving the accurately weighed amount of 20.8 and 18.4 mg of each, respectively, in 100 mL of acetone in a calibrated measuring flask. 10^{-3} M of PA solution was prepared by dissolving the accurately weighed amount of 22.9 mg in 100 mL of chloroform in a calibrated measuring flask.

Standard Solutions

$5x10^{-4}$ M of DES drug was prepared by dissolving 15.54 mg of the drug in 100 mL methanol (in case of DCNP and DNP reagents) or chloroform (in case of PA reagent) in a calibrated measuring flask. Dilute solutions were prepared by accurate dilution from the stock one. All solutions must be protected from light by keeping them in a dark colored quickfit bottles during the whole work and used within two days.

Sample Solutions

Twenty five tablets of DES drug were accurately weighed and the average weight of one tablet was calculated. The tablets were grounded well to a fine powder. A portion of the powder equivalent to 50 mg of the active material was dissolved in 25 mL of methanol or chloroform. The resulting solutions were shaken well for 20 min, filtered through a Whatman No.1 filter paper and washed with the specific solvent. The filtrate and washings of drug were collected in 50 mL measuring flask and completed to the mark with the same solvent.

General procedures

A series of solutions were prepared in which the concentration of reagents was kept constant at 3 mL of DCNP, 2 mL of DNP or 2.5 mL of PA (10^{-3} M) while that of the drug was regularly varied from 0.1 to 2 mL of $5x10^{-4}$ M, then the solution was completed to 5 mL with the suitable solvent. The absorbance of the colored CT complexes were measured at the specific wavelengths (426, 402 and 352 nm) in case of DCNP, DNP, and PA, respectively, against the specific reagent blank prepared similarly without the drug. The linear part of the absorbance-drug concentration graph represents the concentration range within which Beer's law was valid. For more accurate colorimetric analysis; Ringbom plots (Ringbom 1939) had been drawn by plotting log[drug] in $\mu g\ mL^{-1}$ versus percent transmittance and the linear portion of the S-shaped curve gave the accurate range of analysis.

Results and Discussion

The absorption spectra of the reaction products between DES drug (containing secondary amine group) and DCNP and DNP in mixed organic solvents are shown in Fig. 2 and 3. They show characteristic maxima at λ_{max}= 426 and 402 nm. A solution of drug and DCNP in binary system of acetone-methanol (90:10 v/v) yields a golden color with a maximum at λ= 426 nm

(ε^1= 6.14x10^3 L mol^{-1} cm^{-1}), while DNP with DES in acetone-methanol (80:20 v/v) shows two peaks at λ= 370 (ε^1= 12.92x10^3 L mol^{-1} cm^{-1}) and 402 nm (13.720x10^3 L mol^{-1} cm^{-1}). The peak at λ= 402 nm is selected for DES-DNP CT complex studies because it gives the highest absorption intensity as indicated from the ε values, and the reagent showed a relatively weaker absorbance at this wavelength.

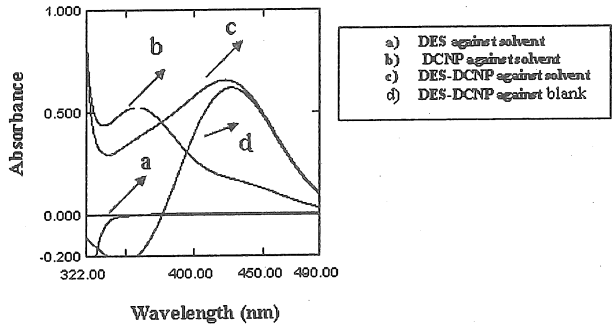


Figure 2. Absorption spectra of CT complex of DES drug with DCNP reagent

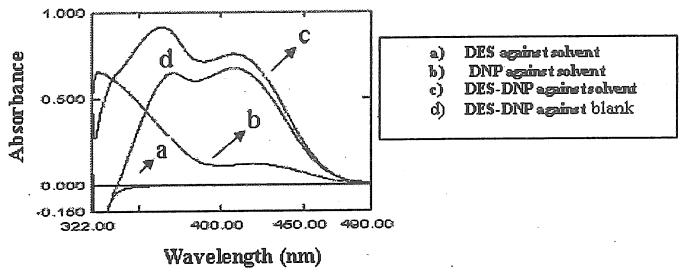


Figure 3. Absorption spectra of CT complex of DES drug with DNP reagent

Fig. 4 shows the absorption spectrum of CT complex of DES with PA in chloroform solvent, which yields an intense yellowish color with two maxima at λ= 352 nm (ε^1= 17.08x10^3 L mol^{-1} cm^{-1}), and 404 nm (ε^2= 12.86x10^3 L mol^{-1} cm^{-1}). This finding may be explained in terms of the formation of a charge-transfer complex between the drug as electron donor and the π-acceptor PA, through the basic nitrogen (in secondary amine). The peak at λ= 352 nm is selected for DES-PA-CT complex studies because it gives the highest absorption intensity as indicated from the ε value, and the peak is more sharp than that of the reagent which shows a relatively weaker band at 404 nm.

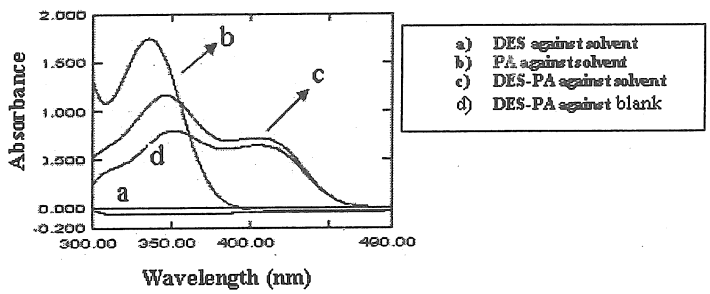
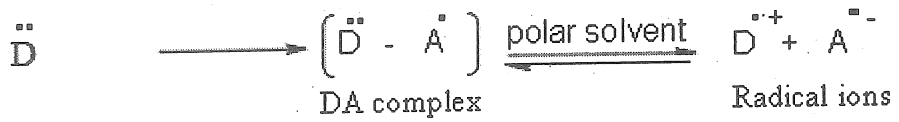


Figure 4. Absorption spectra of CT complex of DES drug with PA reagent

The predominate chromgen with the reagent is the yellow radical anion $A^{\bullet -}$ which is probably formed by the dissociation of an original donor-acceptor (DA) complex with cited drug.



Where D = donor and A = acceptor. $A^{\bullet -}$ = $DCNP^{\bullet -}$ or $DNP^{\bullet -}$

The spectrophotometric properties of the colored CT complexes as well as the different parameters affecting the color development between the drug and each reagent were extensively studied to determine the optimal conditions for the assay procedure. The reaction was studied as a function of reagent volume, nature of the solvent, reaction time and temperature.

Effect of reagents concentrations

To establish the optimum concentration of each reagent, different volumes (0.2–4 mL) of 10^{-3}M DCNP, DNP and PA, respectively, were used. The optimum volumes used for the production of maximum and reproducible color intensity is 3, 2 and 2.5 mL of 10^{-3} mol L^{-1} DCNP, DNP, and PA reagents, respectively, (Fig. 5).

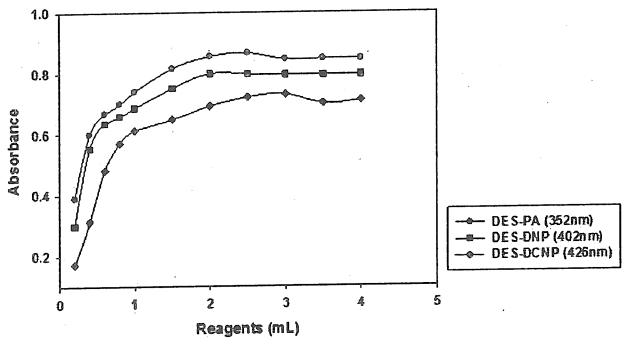


Figure 5. Effect of DCNP, DNP and PA concentrations on the formation of DES CT complexes [reagent]=10^{-3}M

Effect of solvents

In order to select the solvent that would give the highest absorbance, different solvents were tested like acetone, acetonitrile, methanol, ethanol, carbon tetrachloride, 1,2-dichloroethane, dimehylformamide, chloroform, n-hexane, 1,4–dioxane. It was found that, acetone-methanol mixture (90:10 and 80:20 v/v in case of DCNP and DNP, respectively) were considered as ideal solvents as they offer solvent capacity for DCNP or DNP and give the highest yield of the radical anion as indicated by high ε values. This mixed solvent promotes the dissociation of the original CT complex to radical ions i.e. the dissociation of the donor–acceptor complex is promoted by the high ionizing power of the acetone and methanol solvents.

The assay procedures are also applied to DES drug with PA reagent to give CT complex in different solvents. It was found that, chloroform is chosen as the best solvent. But although 1,2-dichloroethane has high molar absorptivity than chloroform but the stability and reproducibility of the absorbance values of the CT reaction is better in chloroform as a solvent.

Effect of time and temperature

The reaction time is determined by following the color development at different time intervals at room temperature under the optimum conditions of the solvent and sequence of addition. It was found that maximum absorbance is attained after 20, 15 and 10 min for the CT formation of DES drug with DCNP, DNP and PA reagents, respectively, and then remain constant up to 60 min. Regarding the stability of the products, it was found to be stable for at least 24 h.

The reaction takes place completely in the presence of the suitable volume of each reagent after the specific time, raising the temperature from (25 to 70°C) does not accelerate the reaction process and does not give reproducible results, so the optimum temperature is the ambient (25±1°C).

Stoichiometry of the CT complexes (Job 1939, Yoe and Jones 1944)

A further study on the CT complexes reaction of DES with DCNP, DNP or PA reagents, the Stoichiometry of the reaction mixture was determined using molar ratio and Job's continuous variation methods (Fig. 6). The results show a 1:1 Drug: Reagents (DCNP, DNP or PA) CT complexes. The CT complexes formed between each reagent and DES drug takes place through the transfer of electron from a donor (drug) to the π-acceptor. Due to the presence of nitro group in DCNP, DNP or PA and two chloro substituents in DCNP, is expected to act as a π-acceptor. The structure of the CT complexes formed between the drug under study and the different reagents is shown in Fig. 7 (Siddiqi and Pathaia 2003).

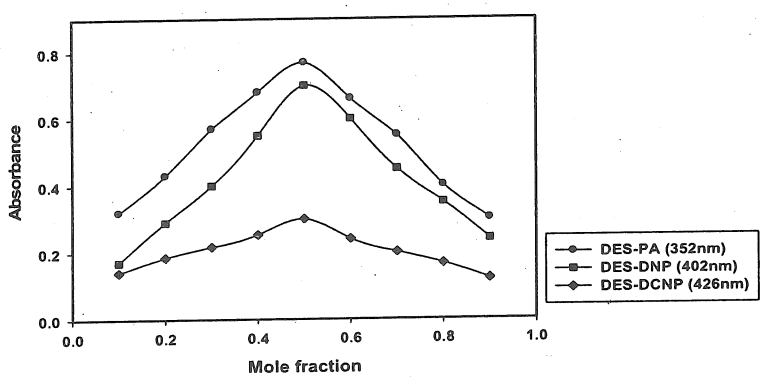


Figure 6. Job's method for DES-CT complexes with DCNP, DNP and PA reagents

Linearity of Beer's law

Under the optimum conditions, Beer's law was obeyed over a very wide range of concentrations using the studied reagents, and was quite suitable for quantitation. Table 1 shows the linear calibration ranges and regression parameters for the proposed methods. For more accurate results, Ringbom optimum concentration ranges were 9.32-62.16, 3.11-49.73

and 3.11-31.08 μg mL^{-1} DES drug and the correlation coefficients are found to be 0.9996, 0.9998 and 0.9999 using DCNP, DNP and PA reagents, respectively.

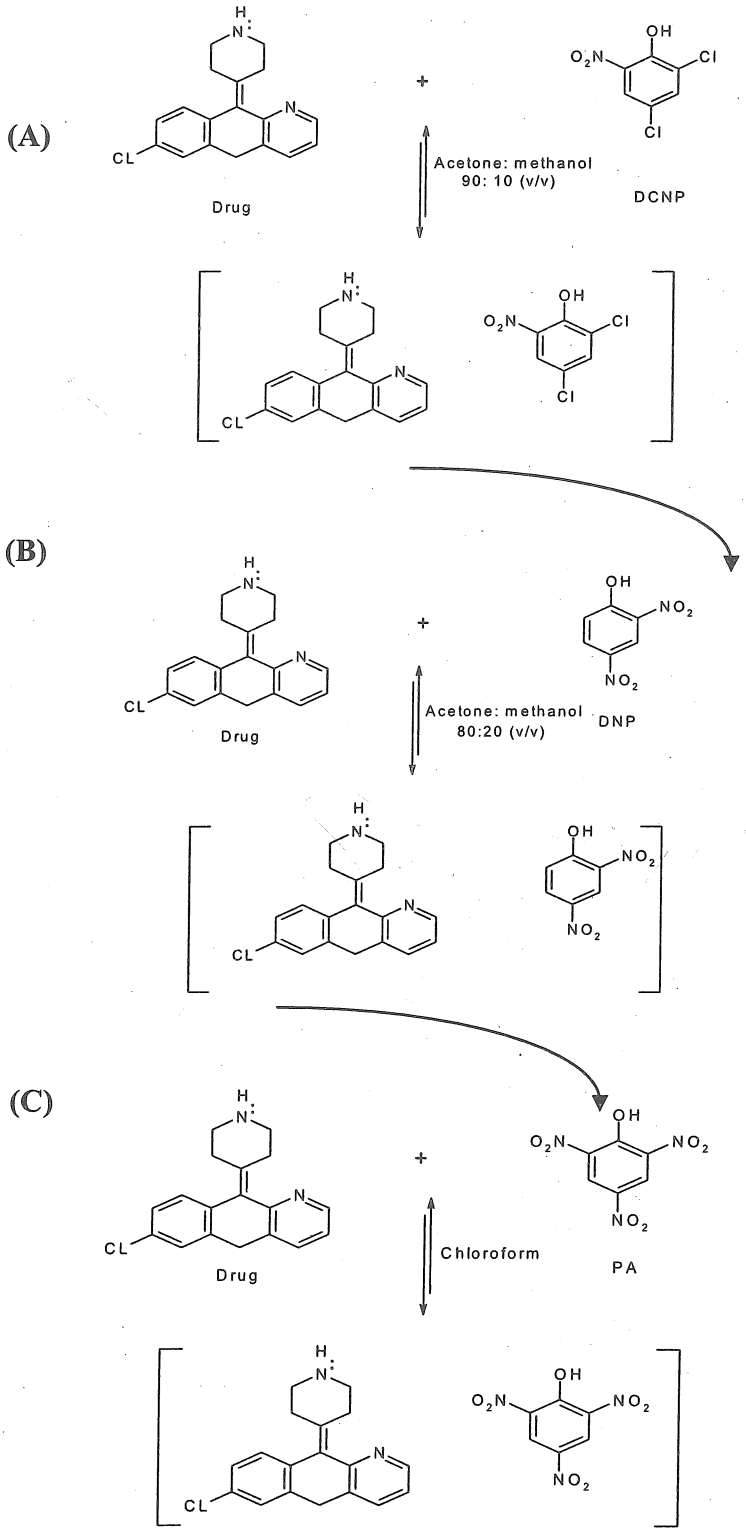


Figure 7. Structures of (A) DES-DCNP, (B) DES-DNP and (C) DES-PA CT complexes

Table 1. Analytical paremeters for the determination of DES drug using DCNP, DNP, and PA reagents

Parameters		DCNP	DNP	PA
λ_{max} (nm)		426	402	352
Concentration Range (µg mL^{-1})		3.11-93.35	3.11-62.17	3.11-43.44
€ (L mol^{-1} cm^{-1})		6.14x10^5	13.72x10^5	17.08 x10^5
Sandell Sensitivity (µg cm^{-2})		0.018	0.023	0.051
A= mC+Z	M	0.0181	0.0860	0.0457
	Z	0.0543	0.1650	0.1808
Correlation coefficient (r^2)		0.9996	0.9998	0.9999
SD		0.018-0.090	0.035-0.123	0.031-0.128
RSD (%)		0.094-0.357	0.155-0.746	0.115-0.514
LOD (µg mL^{-1})		2.132	1.884	0.559
LOQ (µg mL^{-1})		7.107	6.280	1.863
Ringbom limits (µg mL^{-1})		9.32-62.16	3.11-49.73	3.11-31.08

Quantification, accuracy and precision of the proposed method

The validity of the proposed method for the determination of DES was assessed by applying to two commercial forms namely Aerius and Desa tablets. Commercial tablets of DES were successfully analyzed by these methods. Frequently encountered excipients did not interfere, because these reactions are specific for secondary amine. For purpose of comparison, the reported procedure (Delta Pharm, 2005) has been applied; the results are presented in Table 2.

Table 2. Statistical analysis of microdetermination of DES drug in different pharmaceutical preparations using DCNP, DNP and PA compared with the reported method.

Pharmaceutical preparations	Proposed Method			Reported method[16]
	Reagents			
	DCNP	DNP	PA	
1-Aerius tablet				
[DES] taken µg mL^{-1}	20.00	20.00	20.00	20.00
[DES] found µg mL^{-1}	20.03	19.98	20.10	20.08
Mean recovery % ± SD[(a)]	100.2± 0.025	99.9± 0.037	100.5 ± 0.09	100.4 ± 0.053
N	6	6	6	6
t-test (2.23)[(b)]	1.190	1.896	2.111	
F-test (5.05)[(b)]	2.560	2.052	2.884	
2-Desa tablet				
[DES] taken µg mL^{-1}	20.00	20.00	20.00	20.00
[DES] found µg mL^{-1}	19.97	19.99	19.99	19.98
Mean recovery % ± SD[(a)]	99.85 ± 0.055	99.95 ± 0.058	99.98 ± 0.047	99.90 ± 0.088
N	6	6	6	6
t-test (2.23)[(b)]	1.180	2.092	2.093	
F-test (5.05)[(b)]	2.560	2.302	3.506	

[(a)]Mean recovery ± standard deviation of six determinations. [(b)] Values in parentheses are the critical values at P = 0.05. Where: RSD (%) for the proposed method is 0.125-0.170, 0.185-0.193 and 0.169-0.448 for DES drug using DCNP, DNP and PA reagents, respectively. RSD (%) for the reported method is 0.242-0.440 for DES drug.

The proposed method was applied successfully to the determination of DES, either in pure and dosage forms, with good accuracy and precision. Mean recovery is found to be 100.2±0.025, 99.9±0.037 and 100.5±0.09 for Aerius tablet and 99.85±0.055, 99.95±0.058 and 99.98±0.047 for Desa tablet using DCNP, DNP and PA reagents, respectively. As indicated, the assay results obtained using DCNP, DNP and PA reagents were in accord with this obtained by the reported

method. The performance of the suggested method was further judged by Student's *t* and F-tests at the 95% confidence level, the calculated *t*-values did not exceed the theoretical value, which supports that the proposed and reported procedures were equally accurate. In order to prove the validity and applicability of the proposed method and reproducibility of the results obtained, five replicate experiments at three concentrations of DES drug are carried out.

Table 3. shows the values of the between-day relative standard deviations for different concentrations of the drug, obtained from experiments carried out over a period of four days. It is found that, the between day relative standard deviations are less than 1%, which indicates that the proposed method is highly reproducible and DCNP, DNP and PA reagents are successfully applied to determine DES drug via the charge transfer reaction.

Table 3. Between–day precision for the determination of DES drug using DCNP, DNP and PA reagents

Reagents	[DES] taken $\mu g\ mL^{-1}$	[DES] found* $\mu g\ mL^{-1}$	Recovery %	SD	SD %
DCNP	20.00	19.97	99.85	0.057	0.285
	30.00	30.03	100.1	0.032	0.106
	50.00	49.93	99.86	0.097	0.194
DNP	10.00	10.02	100.2	0.043	0.432
	20.00	20.13	100.7	0.079	0.392
	30.00	29.85	99.50	0.099	0.332
PA	10.00	9.99	99.90	0.017	0.173
	15.00	15.06	100.4	0.038	0.254
	25.00	25.06	100.2	0.032	0.126

*The average of five replicates

Conclusion

In conclusion, the proposed methods are simpler, less time consuming and more sensitive. Further, The low values of the calculated standard deviation (SD= 0.02-0.09, 0.04-0.12 and 0.01-0.13 for DES using DCNP, DNP and PA reagents, respectively) and relative standard deviation (RSD= 0.09-0.36, 0.15-0.75 and 0.12-0.52 % for DES drug using DCNP, DNP and PA reagents, respectively), indicate the high accuracy and precision of the proposed method.

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