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Cloud point extraction of vardenafil HCl from pharmaceutical formulations prior to spectrophotometric determination

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Abstract
An eco-friendly cloud point extraction procedure was developed using non-ionic surfactant, Triton X-114, to extract vardenafil HCl (VARD) from aqueous solution. The method was based on the extraction of vardenafil HCl and bromophenol blue from acetate buffer media pH 3.5 to surfactant rich phase (Triton X-114) and formed an-ion pair complex. The extracted surfactant-rich phase was diluted with methanol and its absorbance was measured spectrophotometrically at 420 nm. The effect of different variables such as pH, Triton X-114 concentration, cloud point temperature and time was established. The calibration graph was linear in a wide range of 0.1-2.0 µg mL^{-1} of vardenafil HCl with r^2 =0.9996. The detection limit based on three times standard deviation of the blank (3s) was 20 ng mL^{-1} and relative standard deviation (R.S.D%) is 1.80. The preconcentration factor is 20. The proposed method was applied to the determination of vardenafil HCl in pharmaceutical formulations (tablets).

Keywords: Cloud point extraction; Vardenafil HCl; Spectrophotometry; Triton X-114; Bromophenol blue; Pharmaceutical formulations

Introduction
Vardenafil hydrochloride (VARD) is designated chemically as pipera zine, 1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylimidazo [5,1-f] [1,2,4]triazin-2-yl)-4-ethoxy-phenyl] sulfonyl]-4-ethyl-, monohydrochloride (Figure 1). VARD is widely used as a selective phosphodiesterase type 5- inhibitor (PDE5) in the management of erectile dysfunction [1]. Extensive literature survey revealed that the determination of VARD in pure and dosage forms is not official in any of the pharmacopoeias and therefore, require much more investigation.

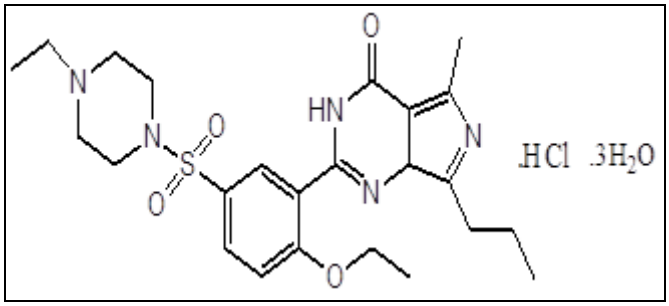

Fig 1: The chemical structure of vardenafil hydrochloride (VARD).

Few reports for the determination of VARD in pure, tablet dosage forms and biological fluids have been developed with the help of a variety of analytical tools including high performance liquid chromatography (HPLC) [2, 11], gas chromatography [12, 13], capillary electrophoresis [14, 15], electrochemical methods [16, 17] and atomic emission spectrometry [18, 20].

All the above methods developed for the quantification of VARD employed complex analytical instruments for their estimation mainly in bulk drug powder, tablet dosage forms and biological fluids. However, most of these methods are complex, require expensive experimental setup and skilled personnel, suffer from time-consuming procedures, and are inaccessible to many laboratories in developing and under developed nations. In contrast, visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure, pharmaceutical formulations and biological samples, due to its simplicity and reasonable sensitivity with significant economic advantages.

To the best of our knowledge, there are some methods have been reported for the quantification of VARD in commercial dosage forms using a spectrophotometric technique [21, 25]. However, these previously reported methods suffer from one or the other disadvantage such as poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid–liquid extraction step; some other methods have a relatively narrow dynamic linear range, involve a heating step, and/or use of expensive reagent or large amounts of organic solvents.

In particular, the traditional liquid-liquid extraction method used large amounts of hazardous, volatile organic solvents. So, in recent years, the green liquid-liquid extraction and cloud point extraction (CPE) have been employed in analytical chemistry. The use of preconcentration step based on phase separation by cloud point technique offers a convenient alternative to more conventional extraction system. Compared with the traditional organic liquid-liquid extraction,

cloud point extraction requires a very small amount of relatively nonflammable and nonvolatile surfactants that are friendly to the environment. The first applications of phase separation based on cloud point phenomenon refer to the extraction of metal ions forming complexes sparingly soluble in water.

Furthermore, surfactant aggregates have been widely used as drug delivery vehicles [26, 27] because they have low viscosity, small aggregate size, simple preparation and long shelf-life. In fact, micellar solubilization is one of the most important properties of surfactant solution, widely used in pharmaceutical, food, detergency and cosmetic industries, enhanced oil recovery and so forth [28].

For organic species, the parameters susceptible to optimization stem from the properties of the surfactant medium that is applied. However, for inorganic species, where the quantitative formation of a hydrophobic complex is an essential prerequisite for efficient CPE, the properties of the surfactant system have to be optimized more carefully, taking into account the variables of complex formation.

Apart from the selection of the appropriate chelating agent, common parameters for both organic and inorganic species, which have to be examined to make CPE successful, are: pH, ionic strength, surfactant type and concentration, temperature, and, equilibrium and centrifugation time [29].

This method is still in its initial stages which means that only few reports on the extraction of environmental pollutants such as polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCBs), vitamins, pesticides and other organic compounds are available in literature [30, 33].

All these indicate that CPE has great analytical potential as an effective enrichment method. To the best of our knowledge, no research has been reported on how to extract VARD through cloud point technique. In this study, we suggest new, simple, sensitive, cost effective and selective spectrophotometric method for the determination of VARD in pharmaceutical dosage forms using a simple CPE process. The method is based on the cloud point extraction of ion pair of VARD-BPB. Triton X-114 was used as an extraction solvent.

2. Materials and Methods

2.1. Apparatus

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm. The pH values of different buffer solutions were checked using an Hanna pH-meter instrument (pH 211) (Romania) equipped with a combined glass-calomel electrode. A centrifuge with 25 mL calibrated centrifuge tubes (Isolab, Germany) were used to accelerate the phase separation process. A thermo stated water bath with good temperature control was used for the CPE experiments.

2.2. Materials and Reagents

All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Materials

Reference standard of pure drugs

Pharmaceutical grade VARD working standard was kindly supplied by their respective manufactures in Egypt, without any conflicts of interests in our submitted paper.

Pharmaceutical formulations

The following tablets were purchased from local commercial markets. Levitra tablets are labeled to contain 10 mg VARD per tablet (Bayer HealthCare Pharmaceuticals, Germany). Powerecta tablets are labeled to contain 20 mg VARD per tablet (Eva Pharma Company Giza, Egypt). Verdenodeb tablets are labeled to contain 20 mg VARD per tablet (Debeiky Pharmaceutical, Cairo, Egypt).

Preparation of standard solutions

Stock standard solutions of VARD (100 μg mL^{-1}) and (1.0×10^{-3} mol L^{-1}) were prepared by dissolving an exact weight (10 mg) of pure drug in least amount of methanol and diluted to 100 mL with the same solvent in a 100 mL measuring flask. The standard solutions were found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use.

Reagent

Bromophenol blue (BDH Chemicals LTD, Poole, England) and used without further purification. Stock solution (1.0×10^{-3} mol L^{-1}) of reagent was prepared by dissolving the appropriate weight of reagent in10 mL of 96% ethanol and diluted to 100 mL with bidistilled water. The dye solution was stable for at least one week if kept in the refrigerator. Acetate buffer solution (CH_3COONa/CH_3COOH) at pH (3.0 - 5.6) was prepared by mixing appropriate volumes of 1.0 mol L^{-1} acetic acid and 1.0 mol L^{-1} sodium acetate solutions [34]. Triton X-114 (tertoctylphenoxy poly (oxyethylene) ethanol) (Fluka, Buches, Switzerland) was used as the non-ionic surfactant without further purification. Aqueous 1.0 % (v/v) solutions of Triton X-114 was prepared by dissolving 1.0 mL of Triton X-114 or Triton X-100 in 99 mL of bidistilled water in 100 mL volumetric flask with stirring.

2.3. General procedures

An aliquot of the standard VARD solution (1000-2000 ng mL^{-1}) were transferred to 10 mL measuring flasks, 2.0 mL acetate buffer pH 3.5, 1.0 mL of thymol blue solution (1.0×10^{-3} mol L^{-1}) then add 2.0 mL of triton X-114 (1.0% v/v) and diluted to the mark with bidistilled water. The resulting solution was transferred to a centrifuge tube and equilibrated at 40° for 15 min. Then the phase separation was accelerated by centrifugation for 5.0 min at 4000 rpm. The mixture was cooled in an ice bath to increase the viscosity of the surfactant-rich phase, and the aqueous phase was easily removed using a syringe pipette. The surfactant-rich phase was diluted with 0.5 mL of methanol. A minimum volume was used for diluting the surfactant-rich phase to measure the absorbance and maximize the preconcentration factor. Then the methanolic surfactant-rich phase was transferred into quartz cell to measure its absorbance at 420 nm against

corresponding reagent blank similarly prepared. The procedures were repeated for other analyte aliquots and calibration plots were drawn to calculate the amount of drug in unknown analyte samples.

2.4. Application to pharmaceutical formulations (tablets)

The contents of twenty tablets of VARD were weighed accurately and ground into a fine powder. An accurate weight of the powdered tablets equivalent to 10 mg drug was dissolved in methanol with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with methanol in a 100 mL measuring flask to give and 100 µg mL^{-1} stock solution of the studied drug for analysis by spectrophotometric method. A convenient aliquot was then subjected to analysis by the spectrophotometric procedure described above. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

3. Results and Discussion

3.1. Absorption spectra

The nitrogenous pharmaceutical compounds are present in positively charged protonated forms and sulphonpthalein anionic dyes present mainly in anionic form at pH ≥ 2.5. So when VARD treated with BPB acid dye at pH range (2.5-5.5) of acidic buffer solution, a yellow ion-pair complex. The VARD - BPB complex was formed by the interaction of the VARD base as n-electron donor and BTB as π-acceptor. The absorption spectra of the ion-pair complex, after CPE process with triton X-114 was measured in the range 350–550 nm against the blank solution. As can be seen, the absorbance at λmax (420 nm) increased after the formation of this complex (Figure 2). Therefore, all measurements were carried out at this wavelength.

The extraction process can be altered by different factors, such as equilibration temperature and time, pH, concentration and nature of the surfactant, and addition of salt. Hence, the effect of these factors on the percentage extraction of the VARD was studied.

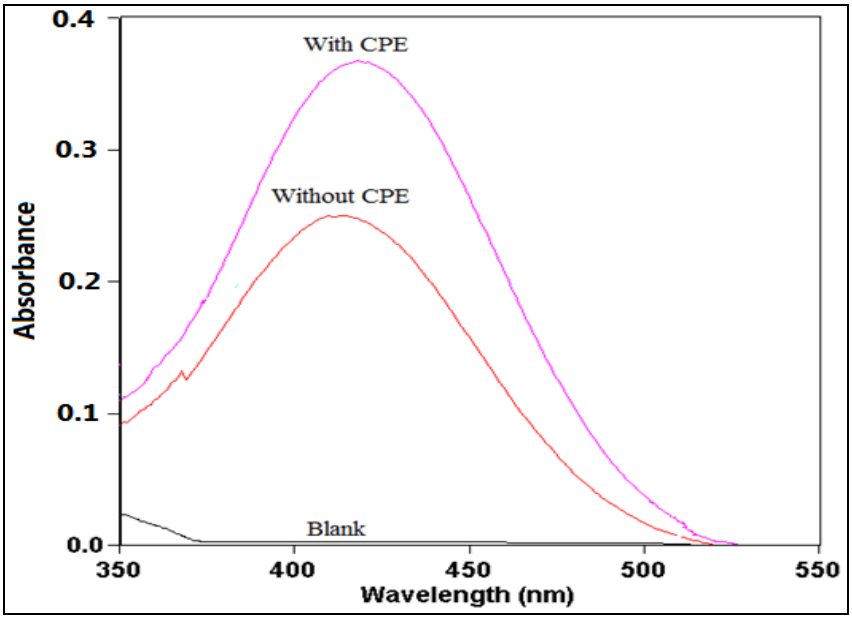

Fig 2: Absorption spectra of the VARD-BPB ion-pair complex with and without CPE process of Conditions: VARD (2.0 µg mL^{-1}), BPB (1.0 x 10^{-3} mol L^{-1}), Triton X-114 (0.2% v/v), pH = 3.5, against reagent blank.

3.2. Effect of pH

For organic molecules, pH is perhaps the most critical factor regulating the partitioning of the target analyte in the micellar Phase. Especially, for ionizable species such as phenols and amines, maximum extraction efficiency is achieved at pH values where the uncharged form of the target analyte prevails. The effect of pH on the extraction of ion pair complex in CPE process was studied for 1000 ng mL^{-1} of VARD in the range of 2.5-5.5 by the addition of appropriate buffer. As can be seen in Figure 3, maximum extraction was obtained at pH 3.5. The extraction equilibria can be represented as follows:

$VARD_{(aq)} + BPB_{(aq)} \leftrightarrow VARD_{(org)} + BPB_{(org)} \leftrightarrow VARDH^{+}\text{-}BPB^{-}_{(org)}$ (ion-pair complex)

Where VARD and BPB represent the drug and the BPB, respectively, and the subscript (aq) and (org) refer to the aqueous and surfactant rich phases, respectively. At pH higher than 3.5, the extraction of BPB and VARD to the organic phase was decreased, hence the formation of ion-pair complex and absorbance was diminished. In addition, at lower pH value the extraction of protonated VARD molecule to the surfactant phase was decreased. Buffer volume was determined by applying the same experiment and variation the volume regularly (0.5-4.0 mL). The maximum extraction efficiency, higher absorbance value and reproducible results were obtained using 2.0 mL of acetate buffer solution.

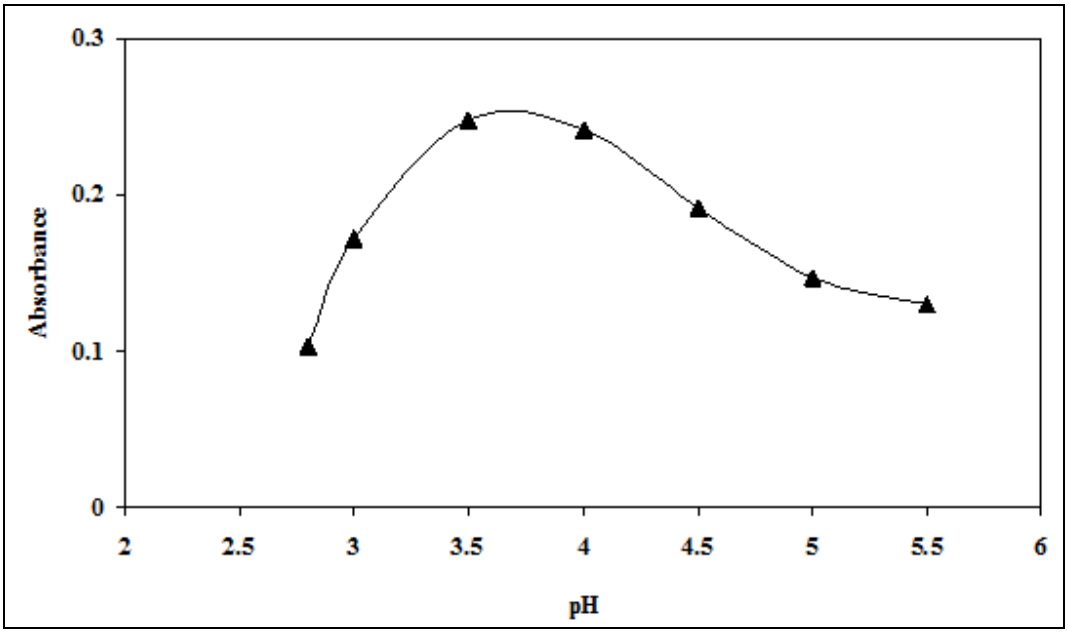

Fig 3: Effect of pH of acetate buffer solution on absorption of the charge transfer complex. Conditions: Triton X-114 (0.2% v/v), BPB (1.0×10^{-4} mol L^{-1}) and 2.0 µg mL^{-1} of VARD.

3.3. Effect of reagent Concentration

The effect of the reagent concentration was studied by measuring the absorbance's of solutions containing a fixed concentration of VARD and varied amounts of BPB reagent. The extraction was studied at different concentrations of BPB and the results are shown in Figure 5. As it is seen, the signal increases up to a known concentration of BPB, reaching a plateau, which is considered as the complete extraction. Maximum color intensity of the complex was achieved with 1.0 mL of BPB reagent (1.0×10^{-3} mol L^{-1}). Although a larger volume of the reagent had no pronounced effect on the absorbance's of the formed charge transferred ion-pair complex (Figure 4).Therefore, a concentration of 1.0×10^{-4} mol L^{-1} was chosen as the optimum amount.

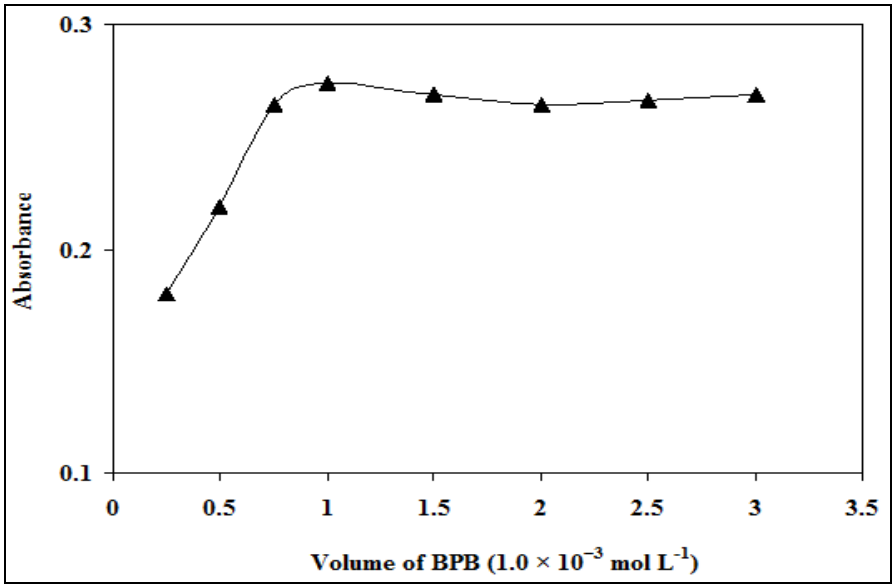

Fig 4: Effect of PBP concentration on absorption of the charge transfer complex. Conditions: Triton X-114 (0.2% v/v), pH 3.5 and 2.0 ng mL^{-1} of VARD.

3.4. Effect of surfactant concentration

The nature of the surfactant used in CPE is a critical factor for its low cloud point temperature (CPT) and high density. Triton X-114 is one of the non-ionic surfactant extensively used in CPE. This is due to its advantages such as commercial availability with high purity, relatively low cloud point temperature, low toxicity and cost and high density of the surfactant-rich phase which facilitates phase separation by centrifugation. Figure 5. shows the effect of non-ionic surfactant concentration within the Triton X-114 concentration range from 0.05–0.5% (v/v), on the CPE efficiency. The absorbance of the complex was increased by

increasing the Triton X-114 concentration up to 0.2% (v/v). A considerable decrease in the absorbance is observed with increasing the surfactant amounts higher than 0.2% (v/v). This can be attributed to an increase in volume and viscosity of the micellar phase. At concentrations below this value, the extraction efficiency of complex was low because there are few molecules of the surfactant to entrap the VARD-BPB complex quantitatively. Thus, Triton X-114 concentration of 0.2% (v/v) was selected for subsequent experiments.

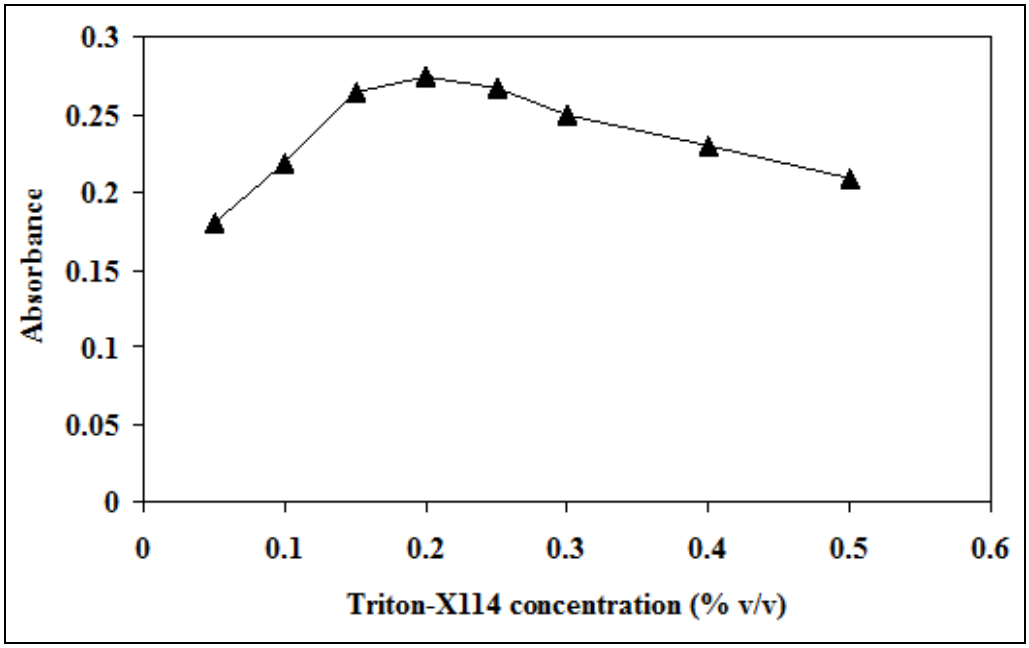

Fig 5: Effect of Triton X-114 concentration on absorption of the charge transfer complex. Conditions: BPB (1.0×10^{-4} mol L^{-1}), pH 3.5 and 1.0 µg mL^{-1} of VARD.

3.5. Effect of added electrolyte

The cloud point of micellar solutions can be controlled by the addition of salts, alcohols, non-ionic surfactants and some organic compounds (salting-out effect). To date, most of studies conducted have revealed that ionic strength has no appreciable effect on the extraction efficiency. An increase in the ionic strength in the CPE does not seriously alter the efficiency of extraction of the chemical forms. Moreover, the addition of a salt can enhance phase separation process [35]. We observed that the addition of NaCl and $NaNO_3$ within the interval of 0.01-0.03 mol L^{-1} had no significant effect on the efficiency. It is not necessary to use additional NaCl or $NaNO_3$ for salting effect because the buffer would have two roles in such a case, one as part of buffering solution for pH control, and the other as salting-out effect.

3.6. Effects of incubation time and temperature

In order to achieve easy phase separation and efficient preconcentration in cloud point extraction processes, it is imperative to optimize the incubation time and temperature. It was desirable to employ the shortest incubation time and the lowest possible incubation temperature, as a compromise between completion of extraction and efficient separation of phases. The influence of the incubation time and temperature was investigated in the ranges 5.0-20 and from 30-60 °C. The results demonstrate that in the incubation time of 10 min and the temperature of 40°C were chosen for further experiments (Table 1). The extraction efficiency for the VARD– BPB complex was constant. Therefore, an incubation temperature of 40°C was chosen for the separation process. Higher temperatures lead to the decomposition of BPB and the reduction of extraction yield. A centrifuge time period of 5.0 min at 4000 rpm was selected as optimum, as complete separation occurred within this time and no appreciable improvements were observed for longer periods.

Table 1: Effect of incubation time and temperature on cloud point extraction of VARD– BPB complex.

Time (min)	Absorbance	Temperature °C	Absorbance
5	0.17	30	0.19
10	0.26	35	0.22
15	0.24	40	0.26
20	0.23	45	0.23
		50	0.22
		60	0.18

3.7. Effects of diluents

In order to decrease the viscosity of the surfactant-rich phase a diluting agent was used. For the spectrophotometric method, the addition of a diluent into the surfactant-rich phase is often needed to obtain a homogeneous solution with compatible viscosity. Methanol, ethanol, acetone and acetonitrile were tested as diluent solvents. Surfactant-rich phase was found to be freely soluble in methanol. Therefore, methanol was chosen in order to have an appropriate amount of sample for transferring and measurement of the absorbance of the sample and also a suitable preconcentration factor. Hence the surfactant-rich phase was completed to 500 µL by methanol. Therefore, the preconcentration factor which defined as the

ratio of the initial solution volume to the volume of surfactant rich phase was 20 using the proposed method.

3.8. Analytical characteristics

The calibration graphs were linear in the range 0.1–2.0 µg mL^{-1} between the absorbance measured and the concentration of the metal in solution were obtained under the optimum conditions of the general procedure. Table 2 summarizes the analytical characteristics such as regression equation, linear range, limits of detection and quantification, reproducibility and preconcentration and enhancement factors.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas [36, 37]:

LOD=3.3σ/s and LOQ=10σ/s

Where σ is the standard deviation of reagent blank, and s is the slope of the calibration curve. In accordance with the formula, the limit of detection and the limit of quantitation were found to be 20 and 67 ng mL^{-1}, respectively.

The precision of the procedure was determined as the relative standard deviation (RSD) and relative error for six replicate measurements carried out in solutions containing 1.0 µg mL^{-1} of VARD were found to be 1.80% and 1.89%, respectively.

The enhancement factor was calculated as the ratio of the slope of the calibration graph with preconcentration CPE procedure to the slope of the calibration graph without CPE was also approximately 15. The consumptive index is defined as the sample volume, in milliliters, consumed to reach a unit of enrichment factor (EF): CI = Vs (ml)/EF was found to be 0.67, where Vs is the sample volume.

Table 9: Optimum conditions and analytical characteristics of the proposed method for determination of VARD with and without CPE.

Parameters	With CPE	Without CPE
λ max (nm)	420	414
Calibration range (µg mL^{-1})	0.1–2.0	1.0–14.0
Molar absorptivity, ε, (L mol^{-1} cm^{-1})	1.3508×10^5	1.0315×10^4
Sandell sensitivity (ng cm^{-2})	4.16	54.44
Regression equation[a]		
Slope (b)	0.0003	0.00002
Intercept (a)	-0.0049	-0.0026
Correlation coefficient (*r*)	0.9996	0.9992
Mean ± SD	100.10 ± 1.80	99.90 ± 2.0
Reproducibility (RSD, %) (*n*=6)	1. 8 (1.0 µg mL^{-1})	2.0 (10 µg mL^{-1})
Limit of detection (LOD) (ng mL^{-1})	20	260
Limit of quantification, (LOQ) (ng mL^{-1})	67	867
t-test [b]	0.76	0.90
F- test [b]	2.88	3.56
Preconcentration factor	20	-
Enrichment factor	15	-
Consumptive index	0.67	-

[a] A = a+ bC, where C is the concentration of VARD in µg mL^{-1}, *A* is the absorbance units.

[b] The theoretical values of *t* and *F* at P= 0.05 are 2.571 and 5.05, respectively.

3.9. Accuracy and precision

Specificity of reaction and selective determination of VARD which was the basic nitrogenous compound with BPB acid dye could be possible. In order to evaluate the precision of the proposed CPE method, solutions containing three different concentrations of VARD were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 3. Lower values of the relative standard deviation (R.S.D%) and percentage relative error (R.E%) indicate the precision and accuracy of the proposed method. The percentage relative error is calculated using the following equation:

$$\%\,R.E. = \left[\frac{found - taken}{taken} \right] \times 100$$

The assay procedure was repeated six times, and percentage relative standard deviation (R.S.D%) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

For the same concentrations of VARD inter- and intra-day accuracy of the method was also evaluated. The percentage recovery values with respect to found concentrations of VARD were evaluated to ascertain the accuracy of the method. The recovery values close to 100% as compiled in Table 2 shows that the proposed method are very accurate. These results of accuracy and precision show that the proposed methods have good repeatability and reproducibility.

Table 3: Intra-day and Inter-day precision and accuracy data for VARD obtained by the proposed method.

Added concentration (µg mL^{-1})	Intra-day				Inter-day			
	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence limit [b]
0.5	99.30	0.61	-0.70	0.496± 0.003	99.0	0.70	-0.60	0.495 ± 0.004
1.0	99.10	0.85	-0.90	0.991 ± 0.009	98.60	0.90	-0.40	0.986 ± 0.01
1.5	99.70	0.90	-0.30	1.495 ± 0.014	100.60	1.40	0.60	1.478 ± 0.022

[a] Mean of six determination, RSD%, percentage relative standard deviation; RE%, percentage relative error.
[b] Confidence limit at 95% confidence level and five degrees of freedom (t = 2.571).

3.10. Recovery studies

To ascertain the accuracy, reliability and validity of the proposed method, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure drugs (50, 100 and 150% of the level present in the tablet) to a fixed amount of drug in tablet powder (pre-analysed) and the total concentration was found by the proposed method. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\% \text{ Recovery} = \frac{[C_F - C_T]}{C_p} \text{x } 100$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drug) added to tablets preparations. The results of this study presented in Table 4 revealed that the accuracy of the proposed method was unaffected by the various excipients present in tablets which did not interfere in the assay.

Table 4: Results of recovery experiments by standard addition method for the determination of VARD in pharmaceutical formulations using the proposed method.

Samples	Taken drug (µg mL^{-1})	Pure drug added (µg mL^{-1})	Total found (µg mL^{-1})	Recovery [a] (%) ± SD
Levitra tablets	0.5	0.5	0.990	99.0 ± 0.70
	0.5	1.0	1.491	99.40 ± 1.0
	0.5	1.5	1.984	99.20 ± 1.30
Powerecta tablets	0.5	0.5	0.985	98.50 ± 0.50
	0.5	1.0	1.470	99.50 ± 0.90
	0.5	1.5	2.020	101.0 ± 1.50
Verdenodeb tablets	0.5	0.5	0.991	99.10 ± 0.80
	0.5	1.0	1.497	99.80 ± 1.40
	0.5	1.5	1.980	99.0 ± 1.60

[a] Average of six determinations.

3.11. Analysis of pharmaceutical formulations

The proposed CPE method have been successfully applied to the determination of VARD in pharmaceutical dosage forms. Six replicate determinations were made. Moreover, to check the validity of the proposed CPE method, dosage forms were tested for possible interference with standard addition method (Table 4). There was no significant difference between slopes of calibration curves and standard addition methods. Therefore it is concluded that the excipients in pharmaceutical dosage forms of VARD were not found any interference in the analysis of VARD. At 95% confidence level the calculated t and F-values did not exceed the theoretical F-value indicating no significant difference between the proposed method and the reported method for VARD [23], (Table 5) [37]. The results show that satisfactory recovery data were obtained and the assay results were in a good agreement with the reported method.

Table 5: Results of analysis of tablets by the proposed CPE method for the determination of VARD and statistical comparison with the reported methods.

Samples	Recovery [a] (%) ± SD	
	Proposed method	Reported methods
Levitra tablets	100.60 ± 0.70	99.92 ± 0.64 (23)
t-value [b]	1.63	
F-value [b]	1.20	
Powerecta tablets	100.70 ± 0.80	99.90 ± 0.67 (23)
t-value [b]	1.70	
F-value [b]	1.43	
Verdenodeb tablets	99.30 ± 0.80	99.50 ± 0.72 (23)
t-value [b]	0.42	

F-value [b]	1.23	

[a] Average of six determinations.

[b] The theoretical values of *t* and *F* are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

4. Conclusion

In this paper, we introduced a novel and sensitive cloud point extraction procedure as a rapid, safe and inexpensive method for the extraction, preconcentration, and determination of VARD spectrophotometrically. The method validation yielded good results and included linearity, repeatability/reproducibility, sensitivity, recovery and accuracy. Triton X-114 was chosen for the formation of surfactant rich phase due to its low cloud point temperature, almost at room temperature. Therefore, the validated method could be useful for routine quality control assay of VARD in raw material and dosage forms (tablets).

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