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Development of an Ecological-friendly Method for Dexamethasone Determination and Cloud Point Extraction in pharmaceutical formulations using Schiff Base Reaction

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Abstract

In this work, new spectrophotometric techniques development for assessment of dexamethasone are described. The first technique including conversion of dexamethasone to colored compound with 2, 4-DNPH as reagent in the acidic medium. The colored compound has a yellow color with absorbance at 405 nm. Between the range of concentration (5.0-40 mg.L^{-1}) , The beer's law is obeyed with correlation coefficient as (0.9959), LOD as 0.507 mg.L^{-1}, LOQ as 1.5 mg.L^{-1}. The second procedure, in technique accompanied by measurement with a UV-Visible spectrophotometer, the CPE technique was used to determine the quantity of the color compound. The linearity of calibration curve between range of (0.5-6.0 mg. L^{-1}), R^2 was 0.9974. LOD and LOQ were found to be 0.108 and 0.354 mg.L^{-1} respectively. This technique was successfully utilized for dexamethasone detection in the several pharmaceutical formulations by REC% was rang between (98-101).

Key word: *Dexamethasone, cloud point extraction, Schiff base, Ecological – friendly and 2, 4-dinitrophenylhydrazin*

1. Introduction

Dexamethasone (DEX) chemically is 9α- fluoro-16α- methyl-11β, 17β, 21-trihydroxy -1,4-pregnadiene-3,20-dione (figure.1) [1]. It is glucocorticoid class of steroids mainly utilized as immunosuppressant and anti-inflammatory [2,3]. This drug is official in the British pharmacopoeia [4], European pharmacopoeia [2], Indian pharmacopoeia [2] and United state pharmacopoeia [5]. In literature, several analytical techniques have been reported for the analysis of dexamethasone in the biological samples such as tears[6], urine [7-11], saliva [12], hair[13], plasma[14-16] , as well as in pharmaceutical preparations [17-19]. The techniques applied to evaluation of DEX in pharmaceutical formulations contain polarographic [20] , UV-Vis spectroscopy [21-24], HPLC [25-28] and HPTLC [29,30]. The sensitive, precise, accurate and reproducible analytical techniques are required to assessment of dexamethasone in the biological and pharmaceutical samples. In this work, new procedure for estimation of DEX using Schiff's base reaction with 2,4-dinitrophenylhydrazine as reagent, then extraction and pre-concentration it utilizing cloud point. Compared to other extraction methods, the cloud point extraction technique has become extremely popular due to the advantage of low organic solvent consumption, quick phase separation, low cost, high recovery and high enrichment. It also cuts down on the time and disposal costs used for the pre-concentration of dexamethasone after Schiff's base compound, which is insoluble in water, is formed [31]. The objective of the current study is to combine and enhance the technique of cloud point extraction with a spectrophotometric technique to determine dexamethasone as a highly sensitive process.

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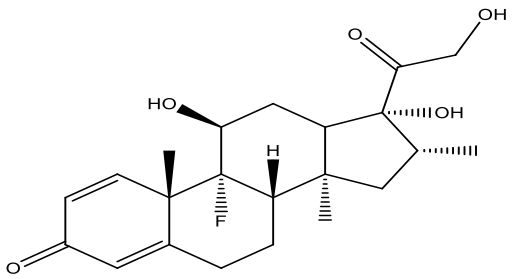

Figure.1: *Structural formula for DEX (M.Wt=392.47)*

2. Experimental

Spectroscopic analysis was achieved on a UV/ VISIBLE 160 single beam UV-Vis spectrophotometer equip with 1.0 cm quartz cell. The pH values were recorded using metlar pH meter. All chemicals & reagent utilized without further purification. Dexamethasone was purchased from the general company for the manufacture of medicines and medical supplies the state company for drugs industry and medical appliances samarra- Iraq and 2,4-dinitrophenyl hydrazine (2,4-DNPH) from Sigma-Aldrich. A stock 2,4-DNPH solution (250 mg/L) was prepared by dissolving 0.025 g of DEX in methanol and diluting to 100 mL. Stock dexamethasone solution (250 mg/L) was prepared by dissolving 0.025g in D.W and dilution to the mark in volumetric flask (100 mL). Stock TritonX-100 was prepared by dissolving 10 g of TX-100 in the 100 mL of D.W.

2.1. Preparation of DEX tablet solution.

A DEX (0.5 mg) 20 tablets supplied from SDI, Samarra, Iraq was powdered and an equivalent amount was transferred to a volumetric flask (100 mL) to prepare a solution (100 $mg.L^{-1}$). The solution was centrifuged for 3 min at 3000 rpm and filtered. The solution was completed to (100mL) using D.W.

2.2. Preparation of DEX syrup solution

50 mL of DEX (5 mg: 5 mL) equivalent to 0.02 g dexamethasone supplied by SDI, samarra, Iraq was put in a volumetric flask (100 mL) and the volume completed with distilled water to the mark to form a solution 200 mg. L^{-1} of DEX drug.

2.3. General procedure of dexamethasone Schiff's base

10 mL of aliquot solution containing (1mL) of DEX (250 mg/L) was mixed with (1mL) of 2,4-dinitrophenyl hydrazine (250 mg/L) and added 3-5 drops of conc.HCl .The solution was complete to the mark with D.W in the 10 mL volumetric flask , then heating the solution for 20 min at 700C. The absorbance of Schiff's base solution was measured at 405 nm against a reagent blank solution treated similarly except without dexamethasone drug.

2.4. Suggested method of cloud point extraction

Various concentrations (0.5- 6 mg/L) of Schiff's base solution of DEX put in the centrifuge tubes (10 mL), also 1.25 mL of TX-100 solution(10%) and distilled water was added to make the volume of solution to 10 mL. The mixture solution kept in the thermostatic bath (70 C) for 50 min. Separation of two phases was achieved by centrifugation (Technik GmbH-Z200 A universal Compact Centrifuge) for 10 min at 4000 rpm. To increase the viscosity of the surfactant-rich phase, the mixture was cooled and the aqueous phase was easily disposed of by decantation. The rich-surfactant phase from this process was diluted with 2 mL of MeOH and put it into quartz cell to measure the absorbance at 405 nm.

3. Results and discussion

The absorption spectra in the acidic media of dexamethasone and its Schiff base compound were assessed as comparison against reagent blank, Figure.2. The Schiff's base compound of DEX was accompanied with red shift of λmax of dexamethasone 405 nm by 200 nm. Investigations to determine the most favorable conditions have been carried out. The influence on the reaction of each of the following variables has been investigated.

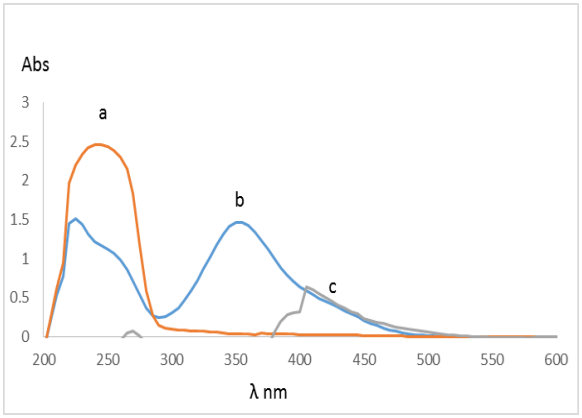

Fig.2: Absorption spectra of a) DEX drug50 $mg.L^{-1}$, b) Reagent 2,4-DNPH, c) Schiff's base compound of DEX

3.1. Optimization of experimental conditions

The experimental conditions were established by investigation of the influence of different parameters such as, effect of solvent, temperature and time of reaction and concentration of 2,4-dinitrophenylhydrazine.

3.1.1. Effect of solvents

The type of solvent utilized influence on the intensity of maximum absorption. Table.1, illustrates the influence of water, methanol, ethanol, dichloromethane and cyclohexane on the absorbance which was very high in the case of using water, methanol and ethanol. On the contrary, the absorbance does not appear in the case of dichloromethane and cyclohexane.

Table.1: Effect of solvent on the absorbance of Schiff's base-DEX formed

Solvent	Abs.
Water	0.435
Methanol	0.421
Ethanol	0.355
CH_2Cl_2	-
Cyclohexane	-

3.1.2. Effect of reaction time and temperature

For DEX drug, the effect of temperature (30-80°C) and time on the condensation reaction is optimized. The results obtained indicate that complete color production is achieved immediately after 20 minutes at 70 ° C (Figure. 3&4). The absorption of the reaction products is increased by a temperature rise of up to 60 °C.

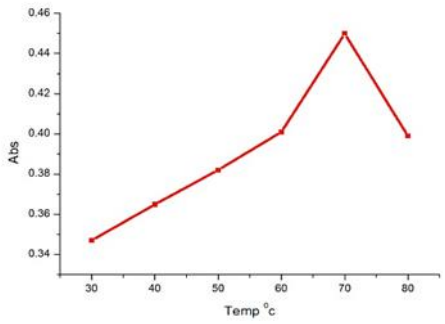

Fig.3: *Effect of* temperature *on the absorbance of Schiff's base-DEX formed*

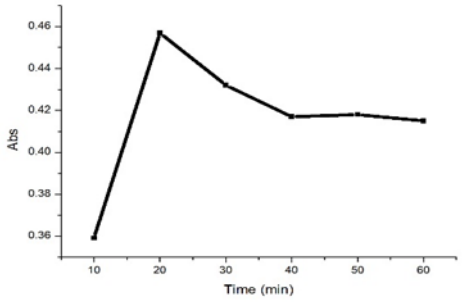

Fig.4: Effect of reaction time on the absorbance of Schiff's base-DEX formed

3.1.3. Effect of amount of coupling reagent

The influence of the amount of coupling reagent has been investigated by adding various volumes (0.25-3.0 mL) 2, 4-DNPH reagent (250 mg.L^{-1}) to the volumetric flask including 1.0 mL of dexamethasone solution (250 mg.L^{-1}) and the volume was complete to 10.0 mL with D.W. Figure. 5 is clear that the volume of 2.25 mL of coupling reagent (250 mg.L^{-1}) is the perfect amount because it gave the greatest absorption, so it is adopted in the next steps.

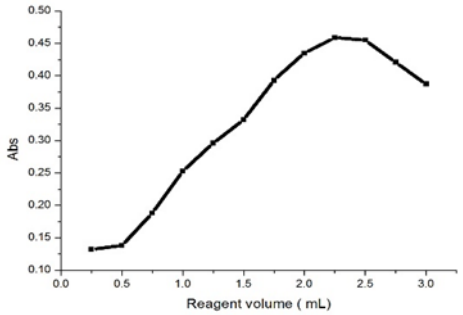

Fig.5: Effect of amount of coupling reagent on the absorbance of Schiff's base-DEX formed

3.1.4. Effect of time on stability of the Schiff's base product

By adding 2.25 mL of 2,4-DNPH (250 mg.L^{-1}) to 1.0 mL of DEX (250 mg.L^{-1}) and complete the volume to 10 mL in a volumetric flask with distilled water, the stability time of the formed colored compound was investigated. The absorption was shown to increase after 5 minutes and the solution remains stable after 20 minutes for at least 40 minutes after dilution and the results are shown in the figure.6.

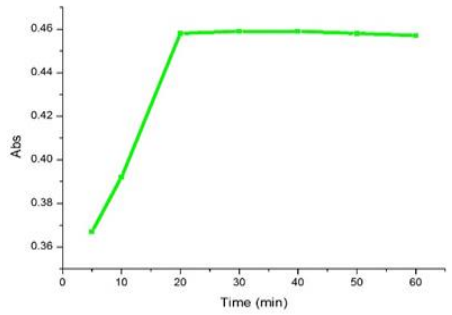

Fig.6: Effect of time on stability of the Schiff's base product on the absorbance of Schiff's base-DEX formed

3.1.5. Procedure for construction of calibration curve

A series of volumetric flask (10 mL), 0.1-0.8 mL of (250 mg.L^{-1}) of dexamethasone are transferred, 2.25 mL of 2, 4-DNPH (250 mg.$L^{-1)}$ and added 3-5 drops of conc. hydrochloric acid, then complete to the mark with distilled water. After that the solution was heated at 70C for 20 min. The absorbance has been recorded at 405 nm against the blank reagent. Figure. 7 illustrate that the calibration curve is linear over the concentration range between 5.0 and 40 mg. L^{-1}, while higher concentrations appear a negative deviation from Beers law.

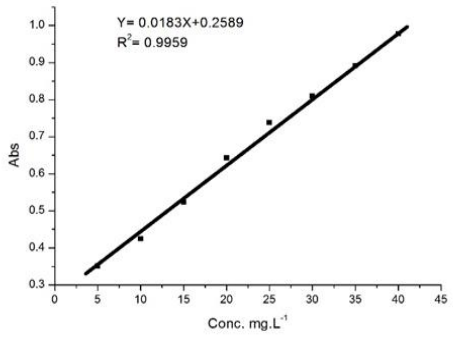

Fig.7: Calibration graph of proposed method of DEX

3.1.6. The nature of formed product

To know the nature of formed Schiff's base product (stoichiometry of DEX with 2,4-DNPH reagent), molar ratio method and job, method have been applied. The concentration solution of each of the standard DEX solution and 2,4-DNPH reagent is equal to 1.2×10^{-3}M. In job,s method, in a series of volumetric flask (10mL) , various volumes of the DEX solution ranging from (0.1-0.9mL) and different volumes of reagent solution(0.9-0.1mL) are mixed, then 3-5 drops of Conc.HCl is added and volumes of solution have been completed with distilled water to the mark. The absorbance was recoded at 405 nm against the blank solution. The results in figure.8 show that ratio is 1:1. In molar ratio method , put the DEX solution in a series of volumetric flask(10mL) are transferred and various volumes of solution of 2,4 DNPH reagent , a few drops Conc. HCl has been added, then volumes have been completed with distilled water to the mark and the absorbance was recorded at 450 nm against the blank solution. Molar ratio was found to be 1:1. The results are shown in figure.9 which is agreement with the job,s method results.

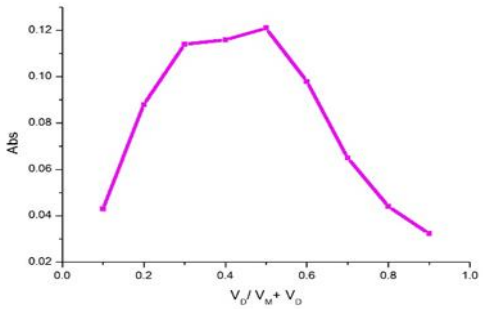

Fig.8: Jobs method of DEX against the reagent 2,4-DNPH

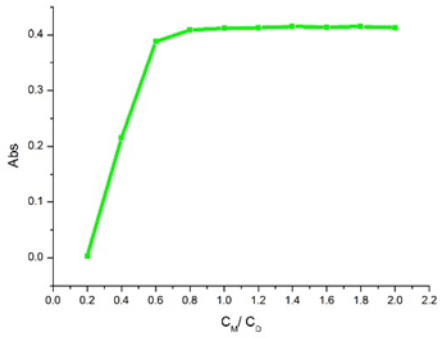

Fig.9: Molar ratio method of DEX against the reagent 2,4-DNPH

3.2. Optimization of cloud point extraction (CPE)

The experiments were achieved to develop a sensitive and simple cloud point extraction technique for determination of DEX drug. After showing a maximum absorption band at 405 nm, the absorption spectrum of Schiff's base compound was reported. To achieve the highest sensitivity, the effect of different parameters on the efficiency of the procedure was studied. In the following equation, the efficiency of extraction is defined:

$$Extraction\ efficiency = \frac{C_S\ V_S}{C_0\ V_0} \times 100\%$$

C_S= Concentration of analyte , V_S= Volume of the surfactant-rich phase, C_0 is Concentration of analyte in the initial sample-surfactant mixture of volume V_0.

3.2.1. Effect type and volume of surfactant

Generally, the extraction is more efficient when more hydrophobic surfactant are utilized. Depend on the literature review, four types of nonionic surfactant have been chosen for optimizing studies, like TritonX-100, Tween80, Tween20 and Sodium dodecyl sulphate(SDS) as a new approach in the CPE. Table.2 show that the tritonX-100 give a highest absorbance and therefore, TX-100 was selected in the subsequent steps. The volume of TX-100 used in CPE is one of the parameters that affect the obtainment of high absorbance. Depend on that, TX-100 volume was studied in the range of 0.25 to 2.0 mL. As shown in the figure.10, the absorbance increased as the volume of TX-100 was from 0.25 to 1.25 mL and decreased at a higher volume of TX-100 surfactant, because the analytical signal deteriorates due to an increase in the volume and viscosity of the surfactant phase [32]. Therefore, 1.25mL of TX-100 surfactant was selected as the optimum condition.

Table.2: Effect Type of surfactant (10%) on the extraction of Schiff's base-DEX formed

Type of surfactant	Abs.
Triton-x100	0.506
Tween 80	0.400
Tween 20	0.129
SDS	-

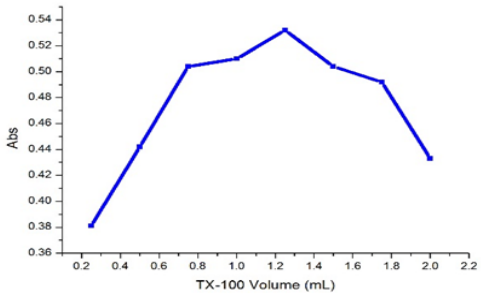

Fig.10: Effect of TX-100 volume on the extraction of Schiff's base-DEX formed

3.2.2. Effect of salt

Ordinary, phase separation in cloud point extraction can usually be done by heating the mixture solution including TX-100 surfactant above the cloud point temperature (CPT). High temperature, however, may lead to analyte losses. The influence of salting-out was implemented as a choice to induce phase separation, depending on this. In this work, to enhance the ability of phase separation in cloud point extraction, the appropriate salts to induce phase separation in CPE were studied. The salts used (1.0 M) were NH_4Cl, Na_2CO_3, NaCl and KCl. The good extraction efficiency was shown by NH_4Cl and therefore, NH_4Cl was chosen as the optimum condition, table.3. The presence of NH_4Cl has a significant effect on reducing surfactant cloud point temperature (CPT) and improving extraction efficiency. The salt volume shows an effect on the absorption because the addition of salt solution will improve the separation of phases. As result in figure.11, various volumes of salt were explored in this study ranging from 0.25 to 1.75 mL . Thus, 1 mL of NH_4Cl was chosen for further study.

Table.3: Effect of salt type (1.0 M) on the extraction of Schiff's base-DEX formed

Salt type	Abs
NH_4Cl	0.533
Na_2CO_3	0.465
NaCl	0.475
KCl	0.103

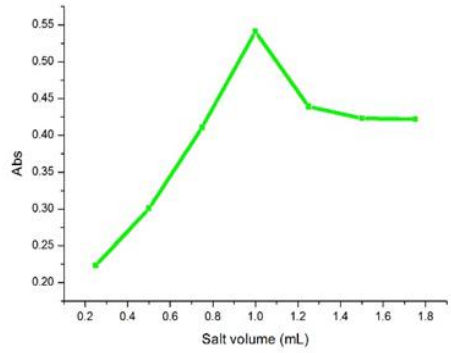

Fig.11: Effect of salt volume on the extraction of Schiff's base-DEX formed

3.2.3. Temperature and incubation time

The temperature effects on the efficiency of DEX extraction are shown in figure. 12. The CMC of nonionic surfactant decreased with temperature, while with the increase of temperature, the number of hydrophobic micelles in the rich-phase surfactant corresponding became higher, leading to an increase in the ability to extract TX-100 surfactant to DEX due

to dehydration in the external layer of micelles [33].Figure.13 shows evidence where the absorbance of DEX increased from 50-70 0C , while beyond 70 0C , the absorbance decreased due to the increases of viscosity. As well as, the effect of incubation time on extraction efficiency was studied in the range of 30 to 70 min. The experimental results shown in figure. 13 indicate that the absorbance of DEX decreased when the time was longer than 50 min. Thus, the extraction time was set at 50 min.

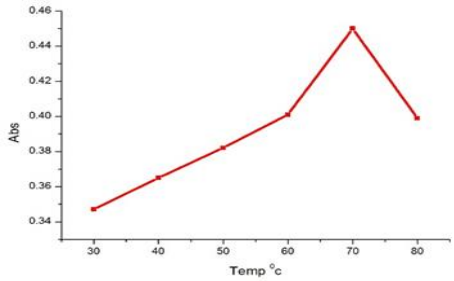

Fig.12: Effect of temperature on the extraction of Schiff's base-DEX formed

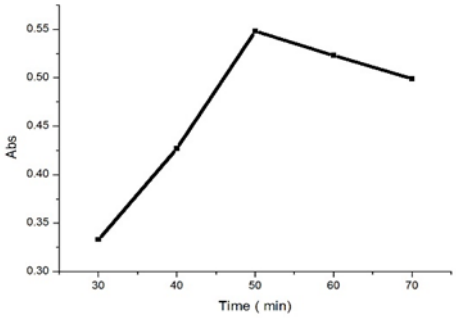

Fig.13: Effect of incubation time on the extraction of Schiff's base-DEX formed

3.2.4. Effect of centrifuge time and rate

Pre-concentrating amount DEX drug with great efficiency in a short time is required. Therefore, cloud point extraction on a set of experiments under optimum conditions by heating to 70 0C and centrifuging at different times and rates and subsequent cooling in 10 min was achieved. Centrifugation at 4000 rpm for 10 min separated the completely two phases table.4 and figure. 14.

3.2.5. Effect of solvent

The influence of various solvents such as MeOH, ETOH, DMSO, H2O and cyclohexane has been investigated for the suggested method, table .5. Methanol gave the highest the absorbance and color intensity of Schiff's base product and it is selected as the ideal solvent.

Table.4: Effect of rotation number on the extraction of Schiff's base-DEX formed

Rotation number (rpm)	Abs
1000	0.337
2000	0.445
3000	0.638
4000	0.644
5000	0.643

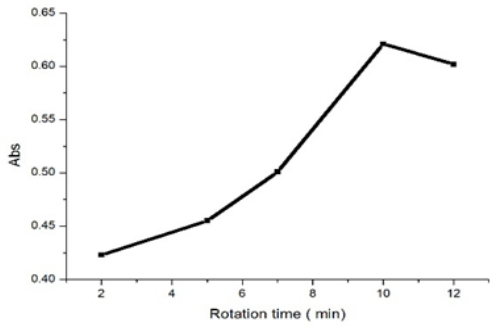

Fig.14: Effect of rotation time on the extraction of Schiff's base-DEX formed

Table.5: Effect of type solvent on the absorbance of Schiff's base-DEX formed

Solvent type	Abs
Methanol	0.678
Dioxane	0.615
Ethanol	0.550
DMSO	0.531
Water	-
Cyclohexane	-

3.2.6. Analytical data of CPE dexamethasone drug

The plotting of dexamethasone concentration versus absorbance (0.5-5.5 mg.L^{-1}) figure.15 and table. 6 was defined under the perfect conditions by the cloud point extraction method for evaluating dexamethasone and linear calibration curve was established.

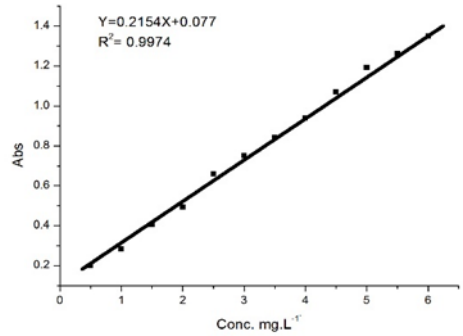

Fig.15: Calibration graph of cloud point extraction method of DEX

3.2.7. Precision and Accuracy

For both methods, the accuracy was estimated by assessment of the percentage, relative error and recovery, while the precision was evaluated the percentage relative error (RSD %), table.7.

4. Conclusion

A simple, accurate, precise, ecological- friendly and sensitive spectrophotometric technique for assessment of dexamethasone drug. The first technique including convention dexamethasone to the colored product (Schiff's base compound) was measured utilizing UV-Vis spectrophotometry. Another technique is cloud point extraction using 2, 4-DNPH as a fairly and stable selective reagent offers a rapid, simple, inexpensive and environmentally benign technique to extract and pre-concentrate in the various samples. This method has a very low detection limit (LOD), good relative standard deviation (RSD %) and was applied to evaluate of dexamethasone drug in the real samples.

Table. 6: Analytical parameter of cloud point extraction method

Parameters	***Before CPE***	***After CPE***
λ_{max} nm	405	
Color	Yellow	
Regression equation	Y=0.0175X+0.2715	Y=0.2154X+0.0877
Linearty range(mg.L^{-1})	5.0-40	0.5-6.0
Correlation Cofficient (R^2)	0.9969	0.9974
Ɛ(L.mol^{-1}.cm^{-1})	2332.2	84536.74
Sandell's sensivity (µg . cm^{-2})	0.1	0.00464
Slope (b)	0.0175	0.2154
Intercept(a)	0.2715	0.077
Limit of detection(mg.L^{-1})	0.507	0.108
Limit of quantification(mg.L^{-1})	1.5	0.354
C.L.for the slope(b±ts$_b$) at 95%	0.0175± 5.463*10^{-4}	0.2154±0.0076908
C.L.for the intercept(a±ts$_a$) at 95%	0.02715±0.0338	0.0877±0.031668
Standard error for regression line ($S_{y/x}$)	0.0213	0.0208
C.L for Conc.X_1 mg.L^{-1}at 95%	19.03±0.0087	0.919±0.022
C.L for Conc. X_2 mgL^{-1}at 95%	39.73±0.098	2.74±0.043
C.L for Conc.X_3 mg.L^{-1}at 95%	58.93±0.135	5.17±0.031

Before CPE (X_1=20, X_2=40, X_3=60) and after CPE (X_1= 1.0, X_2= 2.5, X_3=5.0)

Table.7: Application of the proposed CPE for the evaluation of Dexamethasone

drug	**Before cloud point extraction**					
	Conc. of drug mg.L^{-1}		**Relative Error%**	**Recov. %**	**Average Recov%**	**RSD% (n=5)**
	Taken	Found				
Dexamethasone Tab.	10	10.32	3.2	103.2		2.83
	20	19.97	-0.15	99.85	100.87	0.90
	30	29.87	-0.43	99.56		3.12
Dexamethasone syrup	10	9.60	-4.0	96		5.70
	20	19.93	-0.35	99.65	98.43	2.40
	30	29.90	-0.33	99.66		3.41
After cloud point extraction						
Dexamethasone Tab.	1.0	1.01	1.0	101		4.48
	2.5	2.45	-2.0	98	99.6	4.30
	5.0	5.01	0.2	99.8		1.69
Dexamethasone syrup	1.0	1.02	2.0	98		4.39
	2.5	2.55	0.28	99.9	99.2	3.77
	5.0	4.95	-0.01	99.9		3.33

Table.8: Comparison of the CPE method's LOD and LOQ values with different methods reported in the literature

Method	LOD mg.L^{-1}	LOQ mg.L^{-1}	R^2	Ref.
Spectrophotometric method	0.52	1.56	0.9999	[34]
RP-UPLC method	0.17	0.59	0.9999	[35]
HPLC method	0.13	0.40	0.9997	[36]
UV-Spectrophotometric method	0.0828	0.2512	-	[37]
Spectrophotometric method	0.063	0.19	0.9927	[38]
Kinetic spectrophotometric method	0.14	-	-	[39]
UV-Spectrophotometric method	0.78	2.3	0.999	[21]
First-and third derivative spectrophotometry	0.08	-	0.982	[40]
UV-Spectrophotometric method	0.63	1.9	0.999	[41]
Spectrophotometric and cloud point extraction methods	0.507, 0.108	1.5, 0.354	0.9969, 0.9974	Present work

4. Acknowledgments

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5. References.

[1] Sversut R. A., Vieira J. C., Rosa A. M. , Singh A. K. , do Amaral M. S. , and Kassab N. M., Improved UV Spectrophotometric Method for Precise, Efficient and Selective Determination of Dexamethasone in Pharmaceutical Dosage Forms. Orbital Electron. Journal Chem. 7(1): 5–9(2015).

[2] Dhumal D. M., ShirkhedkarA. A., Nerkar P. P., and SuranaS. J., Simultaneous estimation of moxifloxacin hydrochloride and dexamethasone sodium phosphate in bulk and in ophthalmic solution by RP-HPLC. Journal Chil. Chem. Soc. 57(4):1344–1347(2012).

[3] Karaffa L. S., The Merck index: an encyclopedia of chemicals, drugs, and biologicals. RSC Publishing(2013).

[4] Pharmacopoeia B., The Stationary Office Medicinal and Pharmaceutical Substances (A–I). Vol. III, London, 2567(2009).

[5] Brown W. and Marques M. R. C., 14 The United States Pharmacopeia/National Formulary. Generic Drug Prod. Dev. Solid Oral Dos. Forms. 319(2013).

[6] BaeyensV ., Varesio E., Veuthey J. L., and Gurny R., Determination of dexamethasone in tears by capillary electrophoresis. Journal Chromatogr. B Biomed. Sci. Appl. 692(1): 222–226(1997).

[7] Baranowska I., Markowsk. P., and Baranowski J., Development and validation of an HPLC method for the simultaneous analysis of 23 selected drugs belonging to different therapeutic groups in human urine samples. Anal. Sci. 25(11):1307–1313(2009).

[8] Song L., Bai J., and Zhou W., Determination of betamethasone and dexamethasone in human urine and serum by MEKC after an experimental design. Chromatographia. 68(3): 287–293(2008).

[9] Taylor R. L., Grebe S. K., and Singh R. J., Quantitative, highly sensitive liquid chromatography–tandem mass spectrometry method for detection of synthetic corticosteroids. Clin. Chem. 50(12): 2345–2352(2004).

[10] Zurbonsen K., Bressolle F., Solassol I., Aragon P. J., Culine S., and Pinguet F., Simultaneous determination of dexamethasone and 6β-hydroxydexamethasone in urine using solid-phase extraction and liquid chromatography: applications to in vivo measurement of cytochrome P450 3A4 activity. Journal Chromatogr. B. 804(2): 421–429(2004).

[11] Baranowska I., Markowski P., and Baranowski J., Simultaneous determination of 11 drugs belonging to four different groups in human urine samples by reversed-phase high-performance liquid chromatography method. Anal. Chim. Acta. 570(1): 46–58(2006).

[12] Thijssen J. H., Gispen-de Wied C. C., Van Heeswijk G. M., and Veeman W., Determination of dexamethasone in saliva. Clin. Chem. 42(8): 1238–1242(1996).

[13] Cirimele V., Kintz P., Dumestre V., Goulle J. P., and Ludes B., Identification of ten corticosteroids in human hair by liquid chromatography–ionspray mass spectrometry. Forensic Sci. Int. 107(1–3): 381–388(2000).

[14] Yang Y. et al., Simultaneous quantitation of dexamethasone palmitate and dexamethasone in human plasma by liquid chromatography/tandem mass spectrometry. Journal Chromatogr. B. 862(1–2): 119–124 (2008).

[15] Damonte G., Salis A., Rossi L., Magnani M., and Benatti U., High throughput HPLC-ESI-MS method for the quantitation of dexamethasone in blood plasma. Journal Pharm. Biomed. Anal. 43(1):376–380(2007).

[16] Mandrioli R., Protti M., and Mercolini L., Metabolic syndrome in schizophrenia: focus on the role of antipsychotic medications and indications for therapeutic drug monitoring (TDM) methods. Front. Clin. Drug Res. Obes. 5(5): 1(2020).

[17] Spangler M. and Mularz E., A validated, stability-indicating method for the assay of dexamethasone in drug substance and drug product analyses, and the assay of preservatives in drug product. Chromatographia. 54(5–6): 329–334(2001).

[18] Garcia C. V, Breier A. R., Steppe M., Schapoval E. E. S., and Oppe T. P., Determination of dexamethasone acetate in cream by HPLC. Journal Pharm. Biomed. Anal. 31(3): 597–600 (2003).

[19] Hashem H. and Jira T., Chromatographic applications on monolithic columns: determination of triamcinolone, prednisolone and dexamethasone in pharmaceutical tablet formulations using a solid phase extraction and a monolithic column. Chromatographia. 61(3):133–136(2005).

[20] T. determination of dexamethasone sodium phosphate in pharmaceutical formulations by differential pulse Polarography, “Trace determination of dexamethasone sodium phosphate in pharmaceutical formulations by differential pulse polarography. Anal. Bioanal. Chem. 373(8): 772–776(2002).

[21] Devi G. N. R., V. Prathyusha, Shanthakumari K. , and Rahaman S. A., Development and validation of uv-spectrophotometric method for the estimation of dexamethasone sodium phosphate in bulk and pharmaceutical dosage form. Indo Am. Journal Pharm. Res. 3(7): 5055–5061(2013).

[22] Singh D. K. and Verma R., Spectrophotometric determination of corticosteroids and its application in pharmaceutical formulation. Iran. Journal Pharmacol. Ther. 7(1): 60–61(2008).

[23] Chothani D., Bhalani J., and Vadaliya K. R., Ratio Derivative Uv Spectroscopy Method For Simultaneous Estimation Of Moxifloxacin Hydrochloride And Dexamethasone Sodium Phosphate In Pharmaceutical Dosage Form. Inven. Rapid Pharm Anal. Qual. Assur. (2013).

[24] Bhalani J., Vadalia K., and Dedania Z. R., Validated First And Second Order Derivative Uv Spectrophotometric Methods For Simultaneous Estimation Of Moxifloxacin Hydrochloride And

Dexamethasone Sodium Phosphate In Ophthalmic Dosage Form. Inven. Rapid Pharm Ana Qual Assur. (2012).

[25] Ghanbarpour A. and Amini M., Analysis of Dexamethasone Sodium Phosphate Injection and Ophthalmic Solution by HPLC, Kinetic Interpretation and Determination of Shelf Life. Journal Sci. 6(4): (1995).

[26] Liu H. et al., Separation and determination of dexamethasone sodium phosphate in cochlear perilymph fluid by liquid chromatography with ultraviolet monitoring and electrospray ionization mass spectrometry characterization. Journal Chromatogr. B. 805(2): 255–260(2004).

[27] Kwak H. W. and D'amico D. J., Determination of dexamethasone sodium phosphate in the vitreous by high performance liquid chromatography. Korean Journal Ophthalmol. KJO. 9(2): 79–83(1995).

[28] Gupta V. D., Chemical stability of dexamethasone sodium phosphate after reconstitution in 0.9% sodium chloride injection and storage in polypropylene syringes. Int. Journal Pharm. Compd. 6(5): 395–397(2002).

[29] Sireesha K. R. and Prakash K., HPLC-UV method for simultaneous determination of ofloxacin and dexamethasone sodium phosphate. Int. Journal Pharm. Pharm. Sci. 4, 415–418(2012).

[30] Seid Y., Hymete A., and Bekhit A. A., Application of a stability-indicating HPTLC method for simultaneous determination of chloramphenicol and dexamethasone sodium phosphate in eye drop. Thai Journal Pharm. Sci. 36(3): (2012).

[31] Fahad A. S. , . Thani M. Z, Abdullah A. M., and Dhahi. S. A., Development of an Ecological-friendly Method for Ciprofloxacin Determination and Cloud Point Extraction in Pharmaceuticals using Fe (II)(FeSO4. 7H2O). in IOP Conference Series: Materials Science and Engineering. 871(1): 12028(2020).

[32] Mohd N. I., Zain N. N. M., Raoov M., and Mohamad S., Determination of carcinogenic herbicides in milk samples using green non-ionic silicone surfactant of cloud point extraction and spectrophotometry. R. Soc. Open Sci. 5(4): 171500(2018).

[33] Zhang W. , Duan C., and Wang M., Analysis of seven sulphonamides in milk by cloud point extraction and high performance liquid chromatography. Food Chem. 126(2): 779–785(2011).

[34] Friedrich R. B., Ravanello A., Cichota L. C., Rolim C. M. B., and Beck R. C. R., Validation of a simple and rapid UV spectrophotometric method for dexamethasone assay in tablets. Quim. Nova. 32(4):1052–1054(2009).

[35] Fouad M. M., RP-UPLC method development and validation for simultaneous estimation of vildagliptin with metformin hydrochloride and ciprofloxacin hydrochloride with dexamethasone sodium phosphate. World Journal of Pharmaceutical Sciences. 3(9): 1755-1762(2015).

[36] Dabh. M. J., Patwari A. H., Desai U. H., Doshi D. B., Rathod I. S., and Suhagia B. N., Simultaneous determination of moxifloxacin hydrochloride and dexamethasone sodium phosphate in eye drops by HPLC and absorbance correction method. Journal Chem. Pharm. Res. 4(10): 4462–4467(2012).

[37] Anand K. and Pandey A., Validation Study of Steroidal Drugs (Dexamethasone and Betamethasone) by UV Spectrophotometric Method. Asian Journal Pharm. Clin. Res. 11(7): 501–505(2018).

[38] Pormsila W., Jityongchai D., and Tesphon W., a Modified Quechers Extraction for the Determination of Dexametasone. Eur. Sci. Journal.10(33): (2014).

[39] Akhoundi-Khalafi A. M. and Shishehbore M. R., A new technique for quantitative determination of dexamethasone in pharmaceutical and biological samples using kinetic spectrophotometric method. Int. Journal Anal. Chem. (2015).

[40] Montazeralmahdi V., Sheibani A., and Shishehbore M. R., First-and Third-Derivative Spectrophotometry for Simultaneous Determination of Dexamethasone and Cytarabine in Pharmaceutical Formulations and Biological Fluids. Journal Appl. Spectrosc. 86(5): 843–847(2019).

[41] Sversut R. A., Vieira J. C., Rosa A. M., Singh A. K., do Amaral M. S., and Kassab N. M., Improved UV Spectrophotometric Method for Precise, Efficient and Selective Determination of Dexamethasone in Pharmaceutical Dosage Forms. Orbital Electron. Journal Chem. 7(1): 5–9(2015).