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VALIDATED SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF DICLOFENAC DIETYLAMINE

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ABSTRACT: Simple, sensitive and convenient spectrophotometric methods (method A and method B) are developed for the determination of diclofenac diethylamine (DDEA) in pure form and in formulations. Method A is based on the oxidation of drug by Fe(III) followed by the complex formation between reduced form of Fe(III) with 1,10-phenantroline. The resulting red colored chromogen is measured at 510 nm. Method B is based on the formation of charge transfer complex between DDEA (n-donor) and *p*-chloroanilic acid (π-acceptor) in 1,4-dioxane. The resulting violet colored chromogen is measured at 526 nm. Beer's law obeyed in the concentration range of 2.00-8.00 and 20.00-120.00 µg mL^{-1} for the method A and B, respectively. The molar absorptivity and Sandell's sensitivity are found to be 0.98×10^4, 1.62×10^3 L mol^{-1} cm^{-1} and 3.77×10^{-2}, 2.27×10^{-2} µg cm^{-2} for the method A and B, respectively. The optimum reaction conditions and other analytical parameters are evaluated. Statistical analysis proves that both the methods are reproducible and selective for the determination of DDEA in pure and in dosage form.

INTRODUCTION: Diclofenac diethylamine (DDEA) chemically, *N*-ethylethanamine 2-[(2,6-dichlorophenyl)amino]benzeneacetate **(Figure 1)** is a powerful non-steroidal anti-inflammatory drug (NSAIDs) [1], which has been most commonly used to reduce inflammation and local pain [2] associated with muscle or joint injuries such as sprains, strains, or sports injuries.

It is used for topical application [3] and mainly used to relieve acute pain. DDEA is a prostaglandin synthetase enzyme inhibitor [4] and has many advantages such as less adverse reaction, small dose and little individual difference and so on.

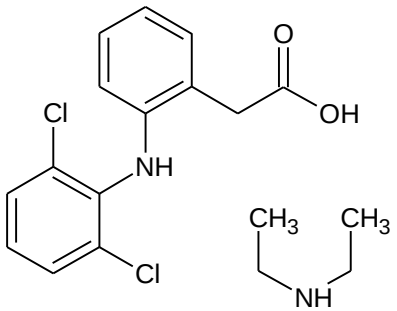

FIGURE 1

It is generally used in addition to other non-medication measures (such as getting enough rest) to relieve these discomforts. Literature survey reveals that many analytical methods are reported for determination of DDEA like HPLC [5-7] and spectrophotometry.

Reported spectrophotometric methods involve the determination of DDEA by using 1% potassium ferricyanide [8] in the presence of 0.5% NaOH and a multiwavelength computational [9].

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The present procedure is advantageous over reported procedures as the reported methods are long, tedious and expensive, involving many reagents and solvents.

MATERIALS AND METHODS:

Apparatus: A UV-visible spectrophotometer (SHIMADZU, Model No: UV 2550) with 1 cm quartz cells was used for the absorbance measurements.

Reagents:

Standard solution of a DDEA: A stock standard solution of 1000 µg mL^{-1} DDEA was prepared by dissolving 0.1 g of pure drug in 100 mL ethanol. The stock solution was diluted approximately to get working concentrations.

Ferric ammonium sulphate (FAS) (0.2%): The aqueous solution of FAS was prepared by dissolving 0.2 g of FAS in minimum volume of conc. sulphuric acid and diluted to 100 mL by using distilled water.

1, 10-Phenanthroline (0.4 %): The solution was prepared by dissolving 0.4 g of the 1, 10-phenanthroline in 100 mL of distilled water.

Chloroanilic acid (CAA) 1%: A stock solution of CAA was prepared by dissolving 0.1 g of CAA in 100 mL 1, 4-dioxane.

Experimental Procedure:

Method A:

Determination of DDEA by using FAS: Aliquots containing (2.00 – 14.00 µg mL^{-1}) of DDEA were transferred into a series of 10 mL volumetric flasks by means of micro burette. To each flask 1 mL of FAS solution and 1.mL of 1, 10-phenanthroline solution were added. The solutions were mixed and kept for 20 min on a water bath at 70°C±1 °C. Resulting solutions were cooled to room temperature and then diluted to 10 mL with 0.1 M H_2SO_4. The absorbance of orange red colored complex was measured at 510 nm against the blank by UV-visible spectrophotometer. The amount of DDEA present in the sample was computed from its calibration curve.

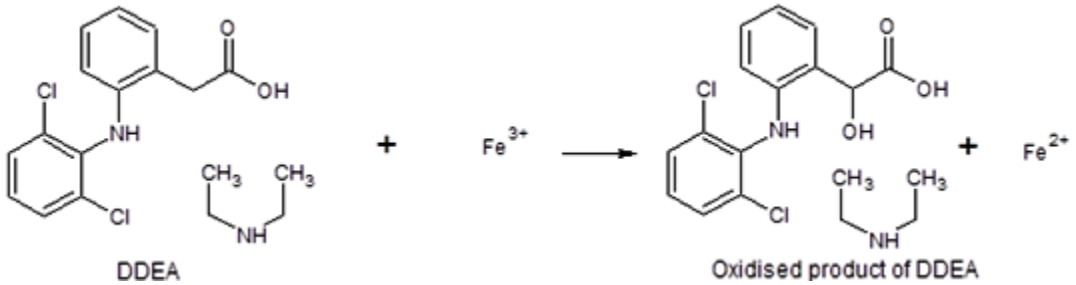
Method B:

Determination of DDEA by using CAA: Aliquots containing (20.00 – 160.00 µg mL^{-1}) of DDEA were transferred into a series of 10 mL volumetric flasks by means of micro burette. To each flask 2 mL of CAA was added. The solution was kept aside for few minutes and the diluted to 10 mL with 1,4-dioxane. The absorbance was measured at 526 nm against the blank. The amount of DDEA present in sample solution was computed from its calibration curve. Alternatively, the corresponding regression equation was derived.

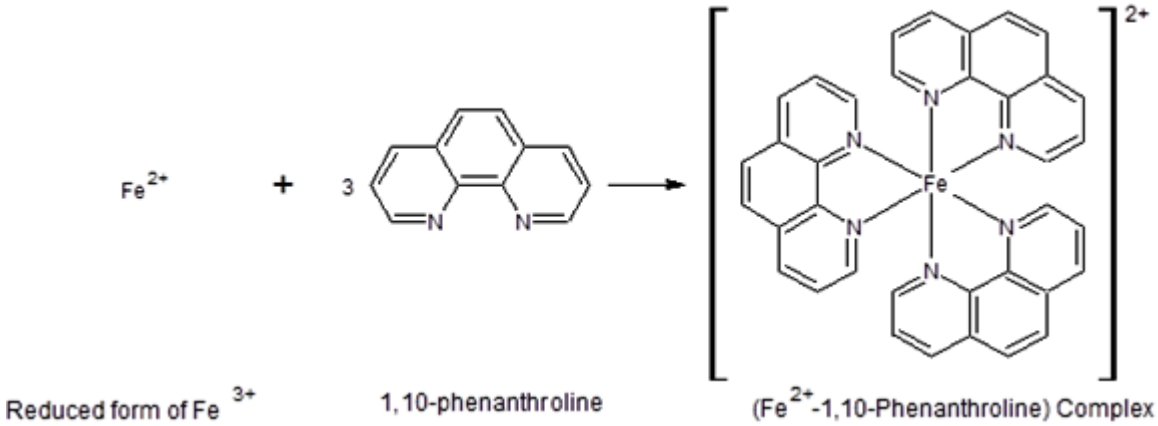
Assay of Formulation: A gel containing 348 mg of DDEA Voveran® Emulgel® was dissolved in 100 mL ethanol [10]. The above solution was filtered using Whatman No. 1 filter paper. The stock solution was diluted approximately to get final concentration of 116 µg mL^{-1}. A convenient aliquot was then subjected to the analysis using the proposed methods.

RESULTS AND DISCUSSION:

Method A: Iron(III) salts play a prominent role in the spectrophotometric determination of pharmaceutical drugs [11]. In this method ammonium iron(III) sulphate acting as an oxidant where iron(III) get reduced to iron(II) and this amount is corresponds to the drug concentration. The amount of iron(II) can be determined by using 1,10-phenanthroline through the formation of ferroin complex **(Scheme 1)** which measured at 510 nm **(Figure 2).**



DDEA Oxidised product of DDEA



SCHEME 1: REACTION OF DDEA WITH Fe(III) FOLLOWED BY THE REACTION OF Fe(II) WITH 1, 10-PHENANTROLINE

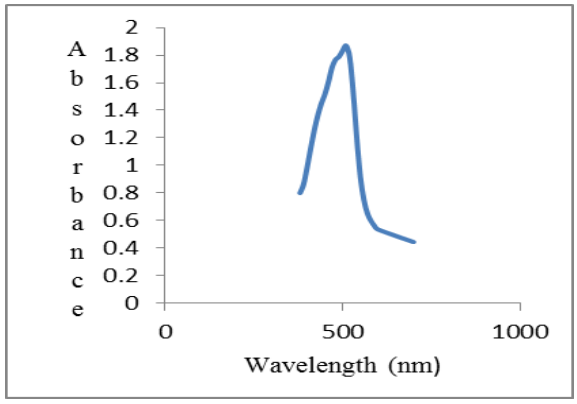
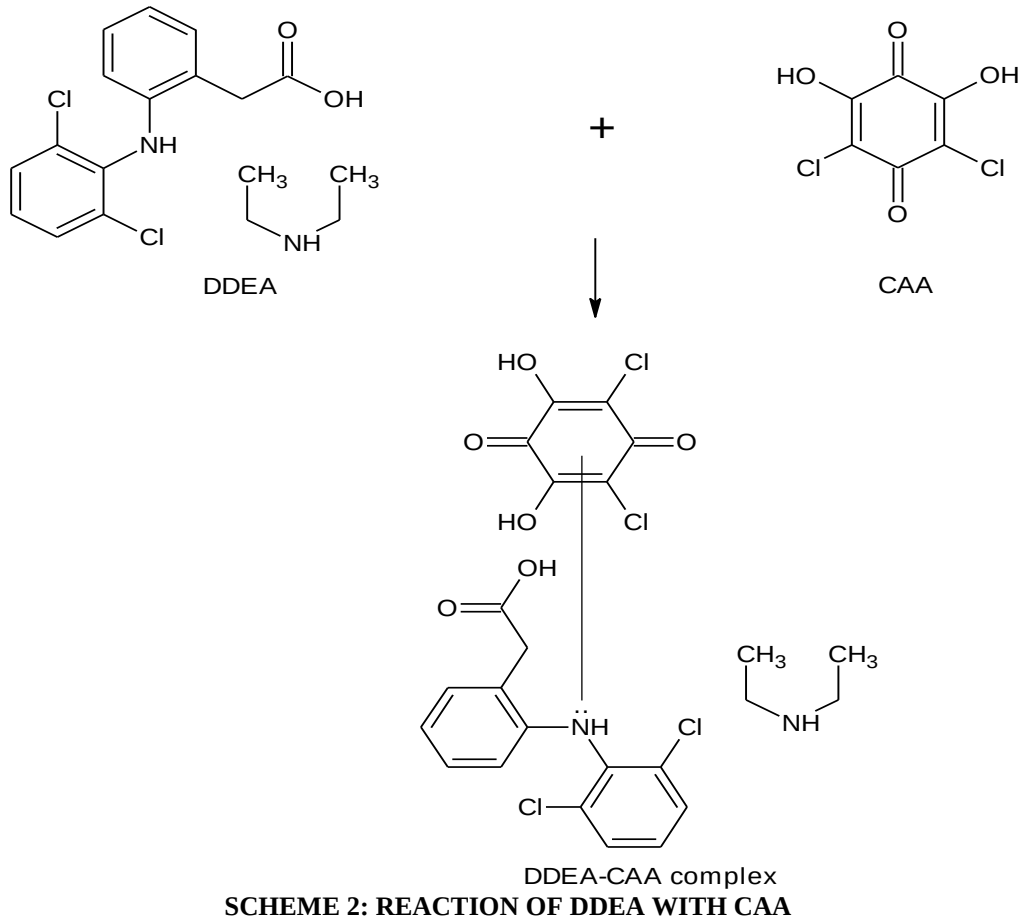


FIGURE 2: ABSORPTION SPECTRUM OF METHOD A

Method B: CAA has been used for the spectrophotometric determination of drugs containing n-electron donors such as nitrogen and oxygen [12]. In this method, the amino group in DDEA serves as n-electron donors and is responsible for the formation of charge transfer complexes with CAA as π-electron acceptor **(Scheme 2)**. CAA in 1, 4-dioxane forms complex with DDEA to form purple colored complex which exhibits absorption maxima at 526 nm **(Figure 3)**. The complex formed is stable for 5 hours.



SCHEME 2: REACTION OF DDEA WITH CAA

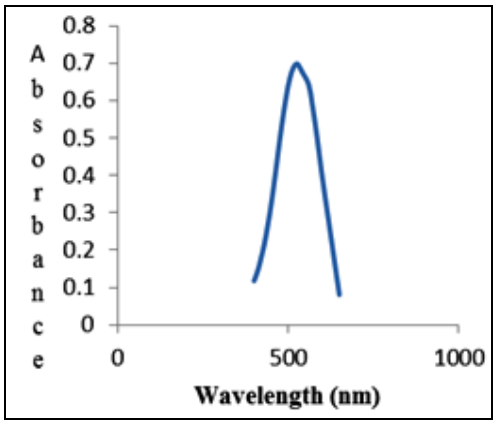


FIGURE 3: ABSORPTION SPECTRUM OF METHOD B

Optimization of Experimental Conditions

1. **Effect of reagent concentration:** The absorbance of the reaction increased with increasing the reagent concentration. It is found that 1 mL of 0.2 % FAS and 2 mL of 1 % CAA is optimal for the formation of colored product.

2. **Effect of temperature:** The reaction between the drug and iron(III) salts in the presence of 1,10-phenantroline is found to be slow at room temperature and required a longer time for completion. Hence, reaction is accelerated by carrying out the reactions at higher temperature. It is found that the maximum absorbance is obtained after heating the reaction mixture at 60°C for 20 min.

3. **Effect of Solvent:** Different solvents like chloroform, 1, 4-dioxane, acetonitrile, ethanol have been tried in order to obtain maximum sensitivity. Among these solvents, 1, 4-dioxane is found to be the best solvent for the formation of stable CAA- DDEA complex.

Analytical performance of developed methods: The methods are tested for linearity, specificity, precision and reproducibility. The calibration graph for the determination of the drug DDEA by the proposed methods are constructed by plotting the absorbance versus concentration (**Figure 4** and **Figure 5**). The linear relationship is obtained over the concentration range given in **Table 1**. The validity of molar absorptivity and Sandell's sensitivity indicates the high sensitivity of the methods. The high value of correlation coefficient (r) obtained for the proposed methods indicate the excellent linearity for the determination of DDEA drug.

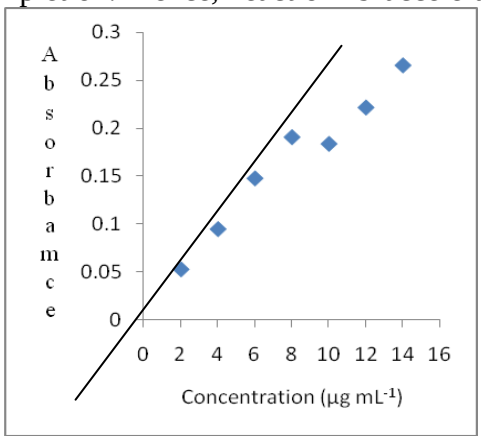


FIGURE 4: BEER'S LAW PLOT OF DDEA

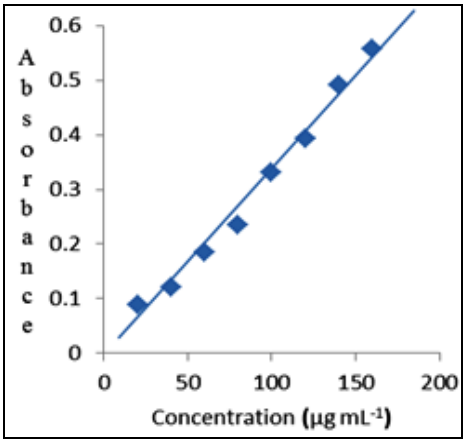


FIGURE 5: BEER´S LAW PLOT OF DDEA FOR METHOD A & METHOD B

TABLE 1: SPECTRAL AND STATISTICAL DATA FOR THE DETERMINATION OF DDEA

Parameters	Method A	Method B
λ_{max} (nm)	510	526
Beer's Law Limits (µg/ml)	2.00-8.00	20.00-120.00
Molar Absorptivity (L mol^{-1} cm^{-1})	0.98 ×1 0^4	1.62 × 10^4
Sandell's Sensitivity (µg cm^{-2})	3.77 × 10^{-2}	2.27 × 10^{-2}
Limit of Detection * (µg mL^{-1})	0.1976	1.2617
Limit of Quantification * (µg mL^{-1})	0.5988	2.9411
Regression Equation**	Y= a + bX	Y= a + bX
Slope (b)	0.0167	0.0034
Intercept (a)	0.0392	-0.0140
Correlation Coefficient (r)	0.9854	0.9933

* Limit of detection calculated according to ICH guidelines. ** Y is the absorbance and X concentration in µg mL^{-1}

Accuracy and Precision: Accuracy is calculated as the percentage recoveries of sample DDEA in their pure form and it is evaluated by standard addition technique at three different concentration levels. Percentage recoveries of both the methods are found to be acceptable.

Precision of the methods are assessed as RSD % at different levels. Reproducibility of the methods is evaluated by the analysis of three different concentrations of the drugs for five replicates. RE and RSD are less than 3% which reveals the high accuracy and precision of the methods. Detailed results are given in **Table 2**.

TABLE 2: EVALUATION OF ACCURACY AND PRECISION

METHOD A

Amount taken (µg mL^{-1})	Amount found* (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
2.00	1.95	2.50	0.27	1.30
4.00	3.92	2.00	0.52	0.13
6.00	5.95	0.83	0.86	0.14

METHOD B

Amount taken (µg mL^{-1})	Amount found* (µg mL^{-1})	RE (%)	SD (µg mL^{-1})	RSD (%)
20.00	19.94	0.30	0.04	0.20
40.00	39.91	0.22	0.40	1.00
60.00	59.90	0.16	0.70	1.32

* Mean value of five determinations. RE - Relative Error; SD - Standard Deviation; RSD - Relative Standard Deviation

The specificity of the methods are investigated by observing that no interference by excipients are encountered which often associated with pharmaceutical preparation. The effect of the presence of some common excipients (starch, lactose, ascorbic acid, citric acid and sodium carbonate) have been studied and optimized.

Applications: Developed methods are quite simple and easy to perform. Therefore it can be successfully applicable for the determination DDEA in dosage form. The content of the pharmaceutical formulation is calculated by applying suitable dilution factor. The results for the pharmaceutical formulation are compared statistically with those of the tabulated t value at 95 % confidence level. The high percentage recovery observed with assay sample in pharmaceutical dosage forms, which indicates that the proposed methods are not affected by interferences from excipients used in formulations (**Table 3**).

TABLE 3: RESULT OF ASSAY OF FORMULATION BY THE PROPOSED METHOD

Brand name	DDEA certified (mg)	Found* ± SD	
		Method A	Method B
Voveran® Emulgel®	11.60	11.63 ± 0.73 t = 0.52	11.46 ± 1.00 t = 0.38

*Mean of five determinations. Tabulated t value at 95% confidence level is 2.77

CONCLUSION: The proposed methods are simple, accurate and can be satisfactorily applied to the analysis of DDEA in bulk and pharmaceutical formulations. The methods are easy to perform and do not contain any intermissive experimental variables, which effect the reliability of the results. There is no interference from common additives and excipients.

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REFERENCES:

1. Kriwet K, Muller-Goymann CC: Binary diclofenac diethylamine-water systems, micelles, vesicles, and lyotropic liquid crystals. Eur. J. Pharm. Biopharm. 1993; 66 (39): 234-38.
2. Suhail A, Safeena S, Patni S: RP – HPLC Simultaneous Estimation of Diclofenac Diethylamine and Lidocaine in Pharmaceutical Gel Formulation. Int. J. Res. Pharm. Sci. 2012; 2(4): 78-88.
3. Hema C, Kanchan K, Saima A, Saurabh A, Vikash K, Sushila R, Permender R: Development and validation of RP-HPLC method for simultaneous estimation of diclofenac diethylamine and curcumin in transdermal gels. J. Liq. Chromatogr. Related. Technol. 2013; 35 (1): 174-87.
4. Bucher U, Sanger A: Diclofenac Emulgel in the Treatment of Localized Rheumatic Disorders, Presented at XVIth Int. Congr. Rheumatol. (IL:AR) Sydney, Australia, 1985; 19–25.

5. Chmielewska A, Konieczna L, Plenis A: Determination of diclofenac in plasma by high-performance liquid chromatography with electrochemical detection. Biomed. Chromatogr. 2006; 20(1): 119-24.
6. Mukherjee B, Mahapatra S, Das S: HPLC detection of plasma concentrations of diclofenac in human volunteers administered with povidone-ethylcellulose based experimental transdermal matrix-type patches. Meth. Fin. Exp. Clin. Pharmacol. 2006; 28(5): 301-06.
7. Arcelloni C, Lanzi R, Pedercini S: High-performance liquid chromatographic determination of diclofenac in human plasma after solid-phase extraction. J. Chrom. B. Biomed. Sci. Appl. 2001; 763(1-2): 195-200.
8. Validya R, Parab RS: Spectrophotometric determination of diclofenac diethylammonium and its dosage forms. Indian Drugs. 1995; 32(1): 194-196.
9. Bucci R, Magri AD, Magri AL; Determination of diclofenac salts in pharmaceutical formulations. Fresenius. J. Anal. Chem. 1998; 362(7): 577-82.
10. Mulgund SV, Phoujdar MS, Londhe SV, Mallade PS, Kulkarni TS, Deshpande AS, Jain KS: Stability indicating HPLC method for simultaneous determination of mephenesin and diclofenac diethylamine. Indian. J. Pharm. Sci. 2009; 7(1): 35-40.
11. Vinay KB, Revanasiddappa HD, Devi OZ, Basavaiah K: Spectrophotometric determination of etamsylate in pharmaceuticals using ferric chloride based on complex formation reactions. Chem. Ind. Chem. Eng. Q. 2010; 16(1): 1-9.
12. Arshiya F, Sayaji R, Venkateshwarlu G: Quantitative determination of Drugs & Pharmaceuticals using p-Chloranilic acid as Reagent. Int. J. ChemTech. Res. 2012; 4(1): 79-91.

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