Estimation of Diclofenac diethylamine and Plumbagin in a drug delivery system by simultaneous equation method

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Abstract

Objective The aim of this study was to develop and validate UV-Vis spectrophotometric method for simultaneous estimation of Diclofenac diethylamine and Plumbagin in a combined dosage form.

Method Solubility studies were performed to select solvent for UV-Vis spectrophotometric analysis. λmax values were selected on the basis of analysis of overlay spectra. Simultaneous equation for estimation of drugs (Diclofenac diethylamine and Plumbagin) in a combined dosage form was developed using value of absorbance and absorptivity.

Key findings Methanol was selected as solvent on the basis of solubility study. Two λmax values, 282nm and 418nm were selected as wavelength of detection. Diclofenac diethylamine and Plumbagin both followed Beer's law over concentration ranges of 5 to 25 µg /ml and 5 to 40 µg /ml, respectively.

Conclusion The simultaneous equation for estimation of Diclofenac diethylamine and Plumbagin was developed according to ICH guidelines and validated for linearity, accuracy, precision, robustness, limit of detection and limit of quantification.

Keywords: UV-Vis Spectroscopy; Simultaneous Equation Method; Diclofenac diethyl amine; Plumbagin

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1 Introduction

Diclofenac diethylamine (DDEA), 2-[2-(2,6-dichloroanilino) phenyl]acetic acid or $C_{18}H_{23}Cl_2N_2O_2$ (Fig.1) is an active pharmaceutical ingredient which belongs to the category of non-steroidal anti-inflammatory drugs (NSAID). It is used to treat low back pain, musculoskeletal problems, strain, sprain, arthritis, contusions (bruises), post-traumatic pain, and inflammation associated with these conditions [1]. It acts by inhibiting the activity of an enzyme known as cyclo-oxygenase (COX) that produces the chemical messenger prostaglandin (PG). As a result, less PGs are formed COX blockage, which helps to reduce pain and inflammation [2].

Plumbagin (PLUM), 5-hydroxy-2-methyl-1,4-naphthoquinone or $C_{11}H_8O_3$ (Fig.1) is a natural anti-inflammatory compound obtained from roots of *Plumbago zeylanica* [3]. Additionally, in the condition of hepatocellular carcinoma, plumbagin specifically blocks p300-dependent acetylation of histone H3, H4, and p53 while having no impact on PCAF (P300/CBP associating factor) indicating its significance as anti-cancer agent [4].

Literature review reveals that numerous analytical techniques are available for estimation of DDEA and PLUM separately [5-11]. However, not a single method for simultaneous estimation of both compounds has been reported till date. Hence, the objective of this study was to establish a quick, accurate, economical, and validated procedure for quantitative determination of DDEA and PLUM in a combined formulation.

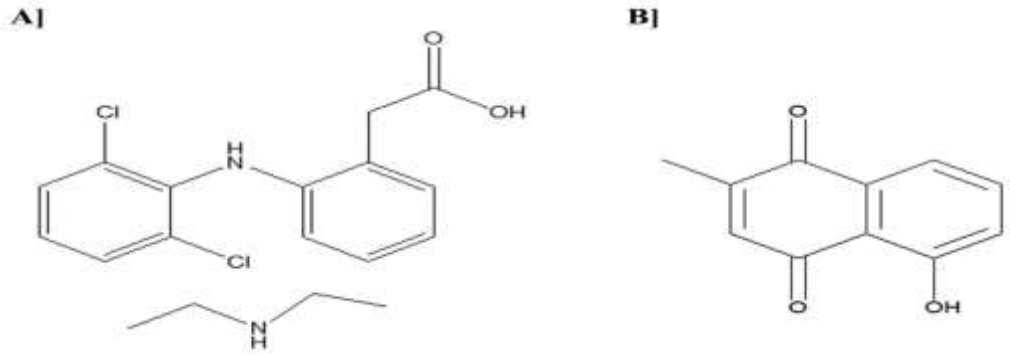

Fig 1. Chemical formula arrangement of (A) Diclofenac diethylamine (B) Plumbagin

2 Materials and Methods

2.1 Materials

Shimadzu UV-1780 double beam UV-Vis Spectrophotometer was used to analyse the spectral data using UV Probe software. Digital ultrasonic cleaner was used to sonicate the prepared solution. APIs were weighed using digital balance (Citizen, Model CDT 220). DDEA was provided as a gift sample from Unique Chemicals (Mumbai, India). PLUM was purchased from P.C. Chem (Mumbai, India). Methanol was procured from Loba Chem Pvt. Ltd (Mumbai, India). All other chemicals were of analytical grade.

2.2 Method development

2.2.1 Selection of solvent

Excess quantity of DDEA and PLUM were dissolved separately in 5ml of each solvent (Water, Acetonitrile, Methanol, Ethanol and Dimethyl sulfoxide) in cyclomixer (CM 101Plus, REMI, India) to determine their solubility. Both drugs showed highest solubility in methanol, therefore selected as solvent for method development.

2.2.2 Preparation of standard stock solution

DDEA and PLUM stock solutions, each comprising 200 µg/ml was prepared by dissolving a fixed quantity (10 mg) of each compound in 50 ml of methanol, followed by 10-minute sonication.

2.2.3 Determination of λmax of DDEA and PLUM

The standard solutions of DDEA and PLUM were further diluted to get final concentration of 10 µg/ml. Each solution was scanned independently between 200–700 nm wavelength range against methanol as a blank. DDEA showed λmax at 282m and PLUM at 418 nm and 265 nm (Fig. 2).

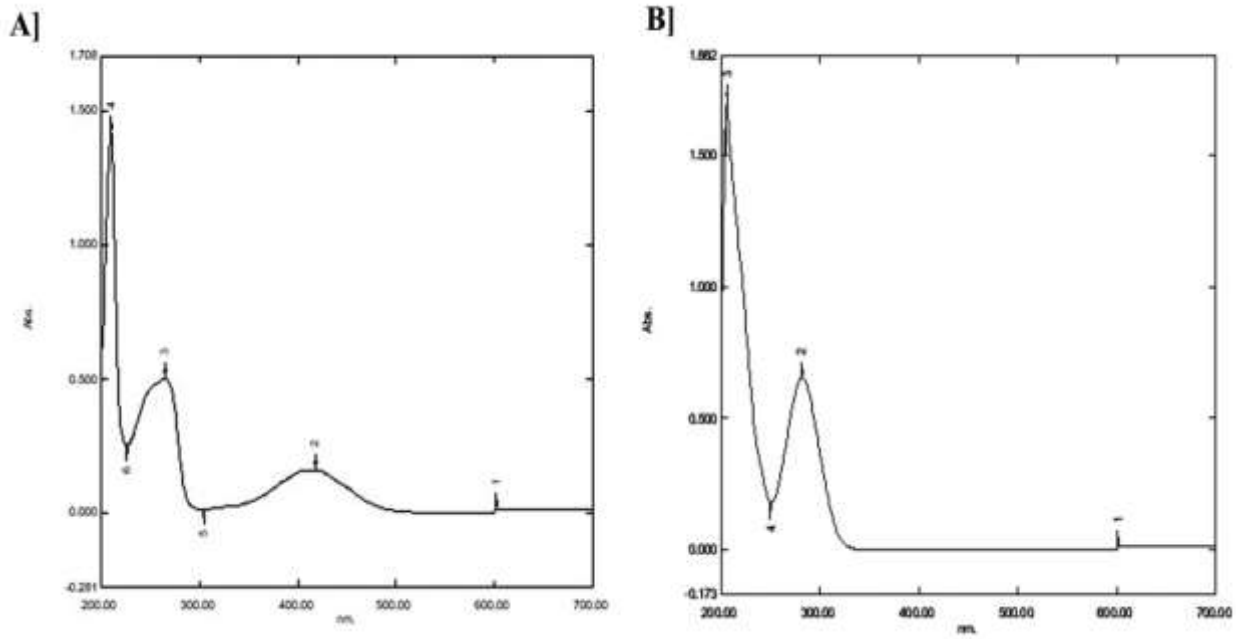

Fig 2. Spectrum of (A) PLUM (B) DDEA

2.2.4 Overlay Spectra of PLUM and DDEA

The overlay spectra of DDEA and PLUM was recorded as shown in Fig.3 and two wavelengths 282nm (λmax of DDEA) and 418nm (λmax of PLUM) were selected for further investigation.

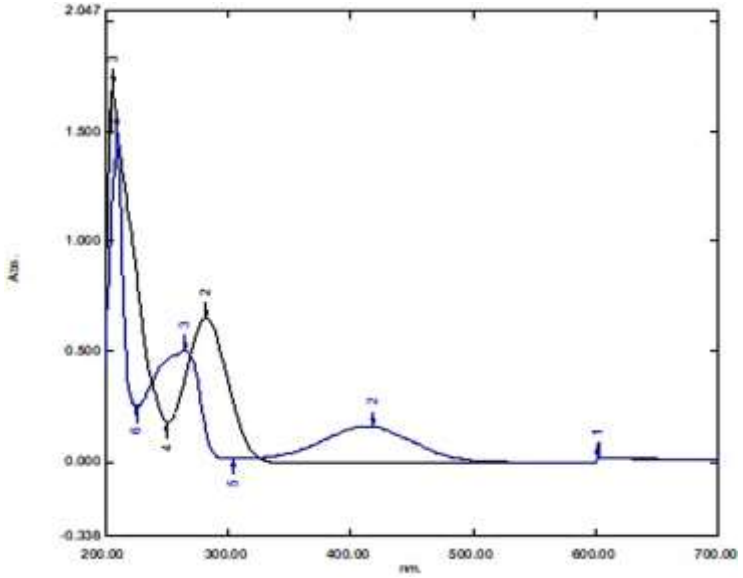

Fig 3. Overlay Spectrum of DDEA and PLUM

2.2.5 Development of simultaneous equation

Standard solutions of DDEA (5 - 25 µg/ml) and PLUM (5 - 40 µg/ml) were prepared separately in methanol and their absorbance values were recorded at 282 nm and 418 nm respectively. To validate Beer's law, the absorbance values were recorded at the appropriate wavelengths and calibration curves were plotted. Absorptivity values A (1%, 1 cm), were calculated using following equation

$$\text{Absorptivity} = \frac{Absorbance}{Concentration\ (gm\ /100\ ml)}$$

Using these absorptivity values simultaneous equation were developed as follows

Cx = A2ay1-A1 ay2/ax2 ay1-ax1 ay2…… (1)

Cy = A1ax2-A2 ax1/ax2 ay1-ax1 ay2…….. (2)

Where Cx and Cy are the concentration of DDEA and PLUM respectively, A1 and A2 are the absorbance of sample solution at λmax 282 nm and 418 nm respectively, ax_1 and ax_2 are the absorptivity of DDEA at 282 nm and ay_1 and ay_2 PLUM at 418 nm [12].

2.3 Validation of developed method

The developed method was evaluated for linearity, accuracy, sensitivity and precision in accordance with ICH Q2B guidelines [13].

2.3.1.1 Linearity

Working standard solutions of DDEA and PLUM were prepared in concentration range of 5 to 25 µg /ml and 5 to 40 µg /ml, respectively, and diluted appropriately before analysis. The least square regression approach was used to calculate the linearity from calibration curves.

2.3.1.2 Precision

Repeatability, intraday, and interday variations were adopted to achieve precision. By analysing the concentrations of DDEA and PLUM at different time intervals under same condition, intra-day precision was estimated. Also the interday precision was determined by analysing the same concentration of solutions on three different days.

2.3.1.3 Accuracy (Recovery studies)

Recovery studies were carried out to examine the accuracy by adding standard drug solutions to pre-analyzed samples at various concentration levels of 80%, 100%, and 120%.

2.3.1.4 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ of developed method were evaluated after examination of three duplicates of same standard solution.

The LOD may be calculated as

LOD = 3.3 × SD/ Slope
The LOQ may be calculated as
LOQ = 10 × SD/ Slope
Where, SD = Standard Deviation of the intercept
Slope = Slope of the calibration curve

2.3.1.5 Robustness

It indicates the ability of a technique to remain undisturbed by minute, intentional changes to the parameters of the method.

2.4 Assay method of gel formulation

The total quantity of drug incorporated in gel was 1 % w/w (0.75 gm DDEA + 0.25 gm PLUM). In order to estimate the concentration of DDEA and PLUM in gel formulation, 0.5 g of formulation was dissolved in sufficient quantity of methanol (5ml) and the required volume was made up to 50 ml in a volumetric flask to get the final concentration of 0.075 mg/ml and 0.025 mg/ml respectively. Further, the solution was sonicated for 20 min to ensure complete dissolution of the DDEA and PLUM. The prepared solution was then analysed spectrophotometrically.

3 Result and Discussion

The developed method was validated in accordance with ICH Q2B guidelines.

3.1 Validation of developed method

3.1.1 Linearity

DDEA and PLUM showed linear response in concentration range of 5-25 µg /ml and 5-40 µg /ml respectively, whereas linear regression equation for DDEA and PLUM was found to be y = 0.074x + 0.1209 and y = 0.0212x + 0.0061 with correlation regression of 0.9961 and 0.9987 respectively. Linearity table and calibration curve has been shown below in Table 1 and Fig.4.

Table 1.Linearity of DDEA

Concentration	Absorbance at 282 nm (n = 3)
5	0.5087
10	0.8280
15	1.2153
20	1.7553
25	1.9440

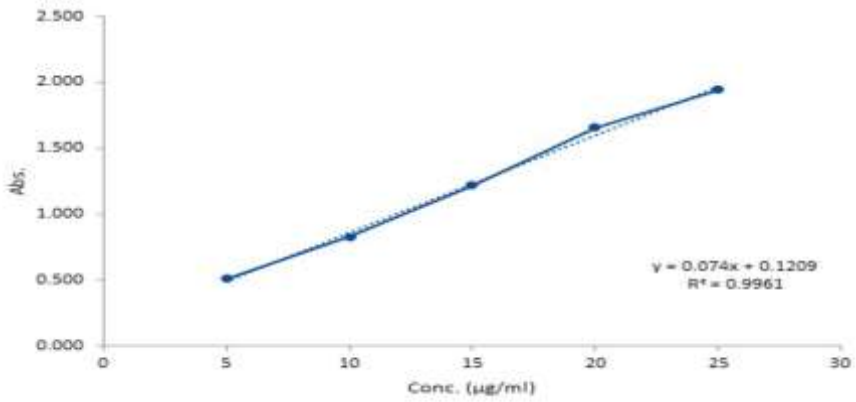

Fig 4. Calibration curve of DDEA

Table 2. Linearity of PLUM

Concentration	Absorption at 418 nm (n = 3)

5	0.110
10	0.235
15	0.312
20	0.423
25	0.539
30	0.642
35	0.760
40	0.851

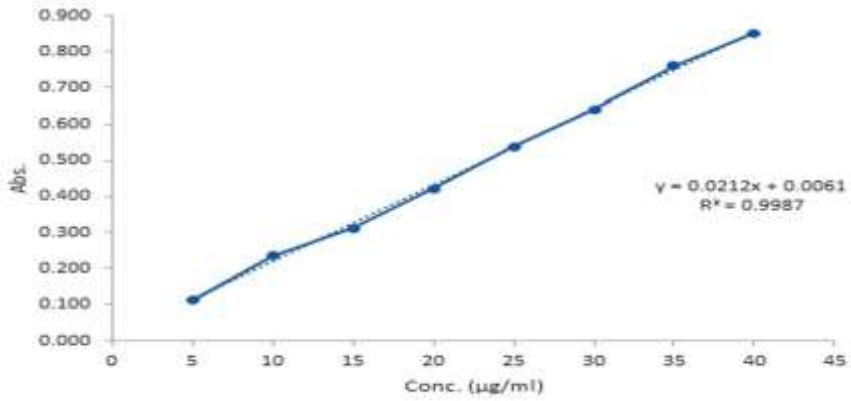

Fig 5. Calibration curve of PLUM

3.1.2 Precision

Repeatability studies, intraday precision as well as interday precision studies were carried out for DDEA and PLUM. The %RSD values were found to be less than 2%. Hence the method so developed was considered as precise (Table 3, 4, 5 and 6).

Table 3. Precision of DDEA (Intraday)

Parameter	Absorption at 0 Hr	Absorption at 2 Hr	Absorption at 4 Hr	Absorption at 8 Hr
DDEA Intraday (n = 6)	1.3354	1.3215	1.3321	1.3215
	1.3242	1.3321	1.3451	1.3568
	1.3520	1.3345	1.3214	1.3211
	1.3440	1.3541	1.3542	1.3112
	1.3200	1.3420	1.3421	1.3211
	1.3452	1.3501	1.3554	1.3102

Mean	**1.3368**	**1.33905**	**1.341717**	**1.320983**
SD	**0.012621**	**0.012119**	**0.013114**	**0.011000**
%RSD	**0.944092**	**0.905023**	**0.977418**	**0.832705**

Table 4. Precision of PLUM (Intraday)

Parameter	Absorption at 0 Hr	Absorption at 2 Hr	Absorption at 4 Hr	Absorption at 8 Hr
	0.1847	0.1911	0.1889	0.1868
PLUM	0.1835	0.1875	0.1869	0.1861
Intraday	0.1876	0.1855	0.1852	0.1844
(n = 6)	0.1901	0.1868	0.1841	0.1834
	0.1865	0.1846	0.1849	0.1865
	0.1871	0.1888	0.1871	0.1878
Mean	**0.186583**	**0.187383**	**0.186183**	**0.185833**
SD	**0.002312**	**0.002344**	**0.001771**	**0.001631**
%RSD	**1.239162**	**1.250835**	**0.951399**	**0.877422**

Table 5. Precision of DDEA (Interday)

Parameter	Absorption in Day 1	Absorption in Day 2	Absorption in Day 3
	1.3354	1.3201	1.3136
DDEA	1.3242	1.3101	1.2994
Interday	1.3520	1.3342	1.3216
(n = 6)	1.3440	1.3226	1.3147
	1.3200	1.3145	1.3201
	1.3452	1.3232	1.3105
Mean	**1.3368**	**1.320783**	**1.313317**
SD	**0.012621**	**0.008285**	**0.007978**
%RSD	**0.944092**	**0.627300**	**0.607456**

Table 6. Precision of PLUM (Interday)

Parameter	Absorbance in Day 1	Absorption in Day 2	Absorption in Day 3
	0.1847	0.1837	0.1866
	0.1835	0.1842	0.1827
PLUM	0.1876	0.1816	0.1854
Interday	0.1901	0.1839	0.1894
(n = 6)	0.1865	0.1857	0.1828
	0.1871	0.1828	0.1841
Mean	**0.186583**	**0.183650**	**0.184000**
SD	**0.002312**	**0.001378**	**0.001684**
%RSD	**1.239162**	**0.750363**	**0.915241**

3.1.3 Accuracy

For DDEA, the **%** mean recovery was found to be 99.75%, 98.98% and 98.54% , whereas for PLUM, 98.44 %, 98.00% and 99.98% at 80%, 100% and 120% levels respectively. The % RSD was found to be less than 2% which indicated the accuracy of method.

Table 7. Recovery of DDEA

Level	Set No.	% Recovery	%Mean Recovery	SD	%RSD
80%	1.	99.81	99.75	1.72	1.72
	2.	101.5			
	3.	98.06			
100%	1.	98.85	98.98	0.16	0.16
	2.	99.15			
	3.	98.9			
120%	1.	98.75	98.54	0.23	0.24
	2.	98.29			
	3.	98.62			

Table 8. Recovery of PLUM

Level	Set No.	% Recovery	%Mean Recovery	SD	%RSD
80%	1.	98.45	98.44	0.01	0.01
	2.	98.42			
	3.	98.44			
100%	1.	97.96	98.00	0.22	0.22
	2.	98.13			
	3.	98.03			
120%	1.	100.05	99.98	0.19	0.19

3.1.4 LOD and LOQ

LOD and LOQ were measured using response and slope of regression. The LOD was found to be 0.80 µg/ml and 2.43 µg/ml and LOQ to be 1.24 µg/ml and 3.77 µg/ml for DDEA and PLUM respectively.

3.1.5 Robustness

Wavelength was altered and checked for the robustness of method. Results obtained showed %RSD less than 2% indicating robustness of developed method.

Table 9. Robustness of DDEA

Parameter	Absorbance at 281nm	Absorption at 282nm	Absorption at 283nm
	1.3254	1.3201	1.3236
DDEA	1.3142	1.3101	1.3194
(n = 6)	1.3239	1.3242	1.3216
	1.3240	1.3226	1.3147
	1.3201	1.3192	1.3201
	1.3250	1.3232	1.3105
Mean	**1.322**	**1.320**	**1.318**
SD	**0.004**	**0.005**	**0.005**
%RSD	**0.326**	**0.391**	**0.367**

Table 10. Robustness of PLUM

Parameter	Absorbance at 417nm	Absorption at 418nm	Absorption at 419nm
	0.1842	0.1837	0.1841
	0.1838	0.1842	0.1839
PLUM	0.1812	0.1816	0.181
(n = 6)	0.1841	0.1839	0.1832
	0.1865	0.1857	0.1862
	0.1822	0.1828	0.1826
Mean	**0.184**	**0.184**	**0.184**
SD	**0.002**	**0.001**	**0.002**
%RSD	**0.997**	**0.750**	**0.943**

3.2 Assay of gel formulation

The drug content of the formulation was found to be 99.84% and 99.33% for DDEA and PLUM respectively.

Table 11. Drug content of formulation

S.No.	% Drug content	
	DDEA	**PLUM**
1.	100.09	99.13
2.	99.77	99.53
3.	99.68	99.35
Mean	99.84	99.33
S.D.	0.215	0.200
%RSD	0.215	0.201

4 Conclusion

A simultaneous equation UV spectrophotometry method for the detection of DDEA and PLUM in combination dosage form was developed and validated in accordance with ICH guidelines. From the aforementioned techniques, a linearity of curve was found, indicating that the solution or dilution followed Beer's law between 5 and 25 µg/ml (DDEA and PLUM). The advantages of the proposed methodology for analytical purposes included quick results, cost effectiveness, simple preparation, good reproducibility, accuracy, and practicality.

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