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Analytical Methods and Permeation Mechanism of Eletriptan

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Abstract: - One of the most popular complaints is headache, specifically migraine. Migraine is a chronic, incapacitating neurovascular situation that causes severe headache the episodes, autonomic nervous system dysfunction, and, in rare instances, an aura with neurologic symptoms. It affects a significant portion of the world's population, with females (15-18%) having a higher incidence than males (6%). Based to a recent WHO evaluation, severe migraine, along with quadriplegia, psychosis, and dementia, is one of the most devastating chronic diseases. There are now two hypotheses clarifying the existence of migraine headache: vascular theory and neural theory. The main goal of migraine therapy is to identify and reduce etiologic or aggravated causes. Furthermore, preventive and abortive therapy may be used to prevent, shorten the length of, and treat symptomologies. As a result, for the administration of an anti-migraine medical care, an approach of delivering the drug into the bloodstream via parenteral route would be ideal. However, the resulting discomfort, danger of infection, difficult administered methods, and the possibility for low patient compliance make such parenteral administration unpleasant.
Keywords: - Eletriptan, Kreb's Solution, Absorbance Values, Headache, Migraine.

Introduction

This estimate approach depends on the finding that eletriptan succinate in 0.1N HC1, distilled water with phosphate buffered saline (pH 6.4), Kreb's solution, sodium free Kreb's the solution, and calcium free Kreb's solution exhibits significant UV absorbance area of the electromagnetic range.

Various analytical techniques for determining eletriptan in plasma and serum samples were established and published, including high performance liquid chromatography (HPLC) with coulometric measurement (Franklin et al., 1996; Dunne et al., 1996).HPLC with florescence detection (Ge Z et ah,2004), and HPLC with mass spectrometric detection (Vishwanathan et ah,2000, McLoughlin et al.,1996, and Dulry et ah,1997). However, no technique for determining eletriptan in brain tissues has been documented to date.

A lot of the procedures mentioned above are highly sensitive, but analytical methods such as HPLC with mass spectophotometry are costly, especially when they need to be estimated in biological samples in regular drug monitoring studies. As a result, a simple, quick, and It is highly recommended that a sensitive method for determining eletriptan in plasma, CSF, and brain homogenate utilizing a new detection technology be developed. An HPLC with UV detection is one of the approaches available. HPLC with UV detection is straightforward, reliable, and widely accessible in analytical laboratories. Although numerous analytical techniques have been proposed, including HPLC with UV/Vis (Shigh et al., 1997, Avadhanulu et al., 1996, Shirsat et al., 1998), To identify eletriptan succinate in the raw product and pharmaceutical formulations; however, to our knowledge, no technique for determining eletriptan succinate has become available. Succinate was detected in biological material using an HPLC with UV detection. Furthermore, the detection limit for the aforementioned analytical procedures utilizing UV detection is substantially greater. An analytical technique for estimating eletriptan succinate in rat plasma, CSF, and brain tissue is designed and validated utilizing important statistical analytical parameters employing HPLC with UV detection. This approach comprises a simple liquid-liquid extraction method with great repeatability, making it suited for eletriptan pharmacokinetics research.

Experimental

Reagents and solutions

(1) 0.1N HC1 was produced according to the procedure outlined in the Indian Pharmacopoeia (1996).

(2) Saline buffered with phosphate (pH 6.4): 1.79 gm disodium hydrogen phosphate, 1.36 gm potassium dihydrogen phosphate, and 7.02 gm sodium chloride in enough distilled water to generate 1000 ml.
(3) Kreb's solution: The Krebs solution (mM) had the following components: NaCl 118, KC1 4.7, CaCh 2.5, MgSCL 1.2, KH2PO4 1.2, NallCCb 22.0, and glucose 11.0.
(4) Sodium-free Kreb's solution: The kreb's solution's sodium chloride and sodium bicarbonate components were substituted with equimolar NMDG (N-methyl D-glucamine).
(5) Calcium-free Kreb's liquid: Calcium chloride was removed from the Kreb's solution, and 2.5mM EGTA was added in its place.

Preparation of calibration curve

Eletriptan succinate stock solution was made in the solvent in which the curve for calibration is to be formed (0.1N HC1, distilled water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution). Sonication was used to dissolve 10 mg of eletriptan in 100 mL of solvent. Pipette suitable aliquots of 100p,g/ml medication stock solution into 10 ml volumetric flasks. The volume was filled with the same solvent, the contents were well agitated, and the amount of absorbance at 283 nm was determined using a Shimadzu UV, 1601 UV-Visible spectrophotometer with cells of 10mm lengths of path over the same solvent used as a blank. The above technique was done six times. Tables 3.1, 3.4, 3.7, 3.10,3.13, and 3.16 show the mean absorbance values and regressed values (method of least squares) of the curves used for calibration in 0.1N HC1, distilled water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution, respectively. In, the calibration curve Figures 3.1,3 3,3.5,3 7,3.9, and 3.11 show 0 IN HCI, distilled water, phosphate buffer sahne (pH 6 4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution, respectively. Tables 3.2, 3.5, 3.8, 3.11, 3.14, and 3.17 illustrate the optical properties of medication solutions in 0.1N HCI, distilled water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution, respectively. The absorption coefficient scans of eletriptan succinate solution in 0.1N HCI, distilled water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution were performed over the emission wavelength range of 200 to 400 nm. Figures 3.2,3.4, 3.6, 3.8, 3 10 and 3.12 illustrate the solutions.

Stability and selectivity

Variations in absorption of eletriptan succinate solutions in 0.1N HCI, distilled water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution utilized for calibration curve preparation at analytical the wavelength of the solution over a 72-hour duration was utilized to evaluate the stability of these solutions with duration.
To gain an understanding of the selection of the newly developed approach for estimating eletriptan succinate, eletriptan succinate was estimated in the presence of other formulation components (chitosan glutamate, carbopol 934P, pluronic-F127) in the same amount in which they were added in the formulations.

Accuracy and Precision

To test the precision as well as accuracy of the approach, known levels of eletriptan succinate in every single one of the previously mentioned solvents were analysed in three replicates using the process described above. The analytical findings from these Tables 3.3, 3.6, 3 9, 3.12, 3.15, and 3.18 describe the findings for 0.1N HCI, distilled water, phosphate buffer salme (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution, respectively.

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Table 1 The calibration curve's mean absorbance values, regressed values, and statistical data for estimating Eletriptan succinate in 0.1 N hydrochloric acid

Concentration (µg/ml)	Mean Absorbance ± SEM*	Regressed Values
2.5	0.025 ± 0.001	0.026
5	0.061 ± 0.002	0.051
10	0.108 ± 0.002	0.100
20	0.200 ± 0.001	0.198
30	0.295 ± 0.005	0.296
40	0.388 ± 0.001	0.394
50	0.471 ± 0.003	0.492
75	0.747 ± 0.009	0.737
100	0.988 ± 0.013	0.982

Regression equation: Y=0 0098X + 0.0029, Correlation coefficient = 0.9991 * Mean of six values

Table 2 Eletriptan succinate optical properties in 0.1 N hydrochloric acid

Optical properties	Value
Absorption maxima	283
Beer's law limit at 283 nm (µg/ml)	2.5-100
Apparent molar absorptivity at 283 nm (1 mol^{-1} cm^{-1})	$4.46x10^{3}$
Sandell's sensitivity coefficient (S) at 283 nm (µg/cm^2/0.001 abs unit)	$9.27x10^{-2}$

Table 3 The accuracy and precision of the Eletriptan succinate measurement technique in 0.1 N hydrochloric acid were evaluated.

Added (µg/ml)	Found (µg/ml) ± SD[a]	Coefficient of variation (CV)	% Relative mean error	Confidence limits[b]
5	5.52 ± 0.10	2.00	2.245	5.52 ± 0.253
20	19.64 ± 0.33	1.67	1.820	19.64 ± 0.816
40	38.85 ± 0.46	1.18	2.866	38.85 ± 1.144
70	74.57 ± 1.17	1.57	0.576	74.57 ± 2.918

an=3, b Confidence limits at P=0 95 and two degrees of freedom.

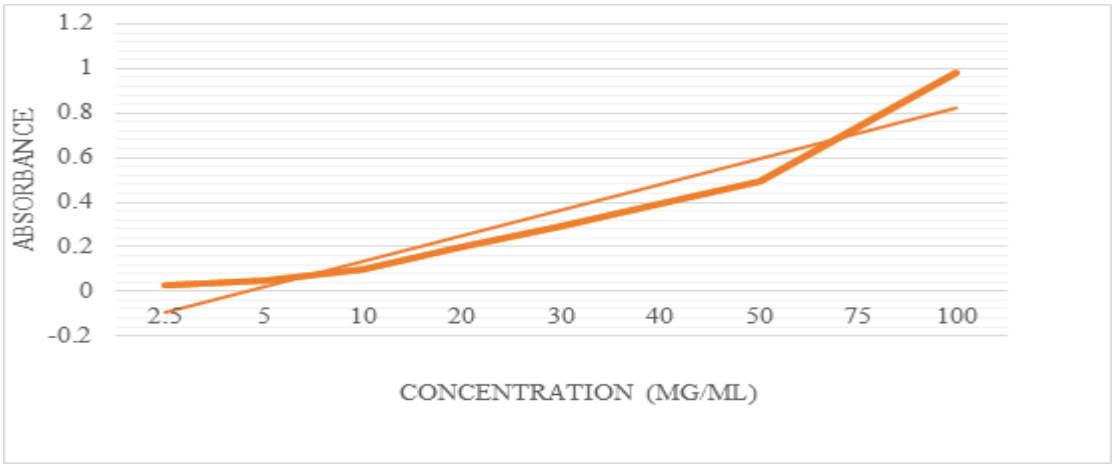

Figure 1 Calibration curve of eletriptan succinate in 0.1N HC1

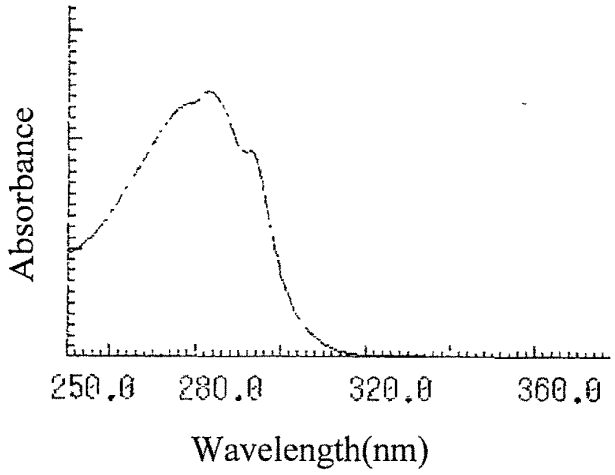

Figure 2 Absorptivity scan of eletriptan succinate in 0.1N HC1

Table 4 Mean absorbance values, regressed values, and statistical data from the calibration curve for Eletriptan succinate estimation in distilled water.

Concentration (µg/ml)	Mean Absorbance ± SEM*	Regressed Values
2.5	0.032 ± 0.003	0.027
5	0.054 ± 0.002	0.051
10	0.115 ± 0.006	0.102
20	0.185 ± 0.004	0.197
30	0.282 ± 0.004	0.297
40	0.383 ± 0.008	0.393
50	0.487 ± 0.008	0.491
75	0.736 ± 0.014	0.733
100	0.971 ± 0.020	0.976

Regression equation: Y=0.0097X + 0.0037, Correlation coefficient = 0.9993 * Mean of six values

Table 5 Optical Characteristics for Eletriptan succinate in distilled water

Optical properties	Value
Absorption maxima	283
Beer's law limit at 283 nm (µg/ml)	2.5-100
Apparent molar absorptivity at 283 nm (1 mol^{-1} cm^{-1})	$4.75x10^3$
Sandell's sensitivity coefficient (S) at 283 nm (µg/cm^2/0.001 abs unit)	$8.71x10^{-2}$

Table 6: Evaluation of the accuracy and precision of the Eletriptan succinate estimation technique in distilled water.

Added (µg/ml)	Found (µg/ml) ± SD[a]	Coefficient of variation (CV)	% Relative mean error	Confidence limits[b]
5	5.08 ± 0.10	2.01	1.645	5.08 ± 0.2560
20	19.52 ± 0.43	2.67	1.420	19.52 ± 1.1753
40	39.58 ± 0.56	1.28	1.066	39.58 ± 1.3140
75	74.98 ± 0.87	1.17	0.076	74.98 ± 2.0496

$a^n=3^b$ Confidence limits at P=0.95 and two degrees of freedom

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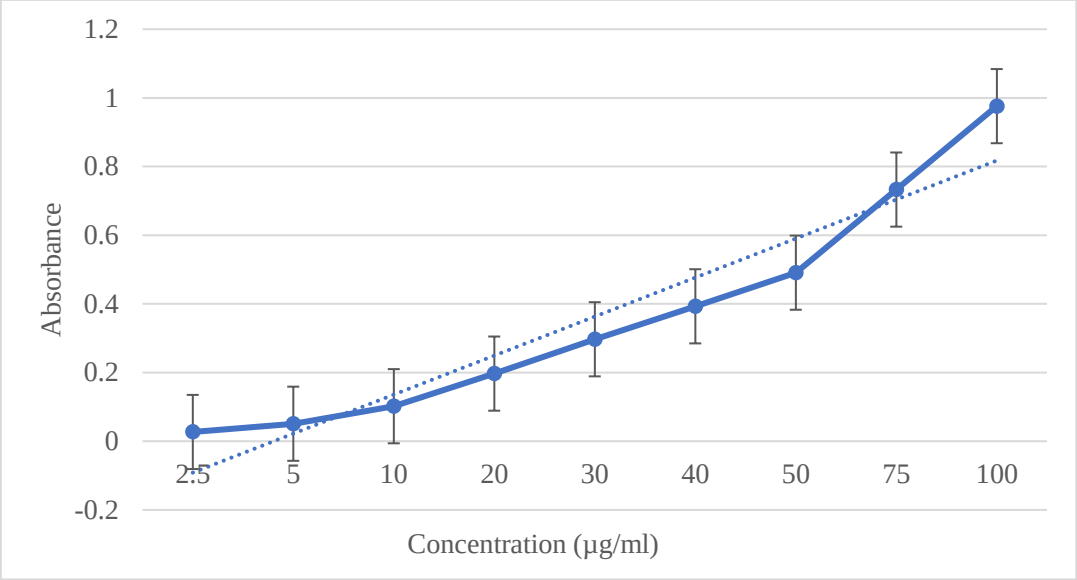

Figure 3 Calibration curve succinate in Distilled water

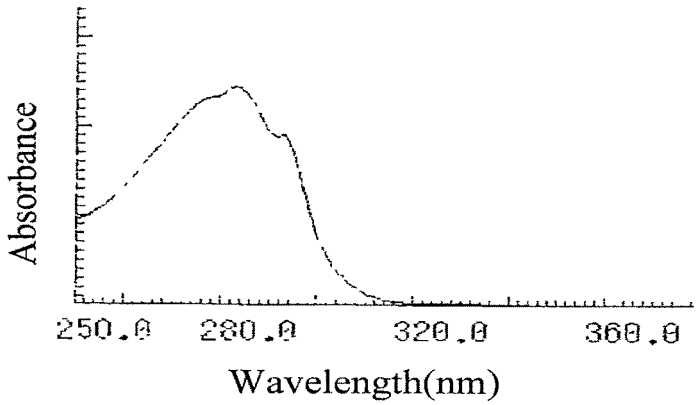

Figure 4 Absorptivity scan of eletriptan succinate in Distilled water

Table 7 Mean absorbance values, regressed values, and statistical data from the calibration curve for Eletriptan succinate estimation in Phosphate buffered saline (pH=6.4).

Concentration (µg/ml)	Mean Absorbance ± SEM*	Regressed Values
1.25	0.020 ± 0.001	0.020
2.5	0.034 ± 0.002	0.036
5	0.065 ± 0.002	0.068
10	0.135 ± 0.003	0.128
20	0.242 ± 0.004	0.248
30	0.353 ± 0.007	0.369
40	0.482 ± 0.005	0.486
50	0.597 ± 0.004	0.606
75	0.895 ± 0.012	0.907
100	1.215 ± 0.002	1.206

Regression equation- Y=0.012X + 0 0038, Correlation coefficient = 0 9997 *Mean of six values

Table 8 Optical Characteristics for Eletriptan succinate in Phosphate buffered saline(pH=6.4)

Optical properties	Value
Absorption maxima	283
Beer's law limit at 283 nm (µg/ml)	1.25-100
Apparent molar absorptivity at 283 nm (1 mol^{-1} cm^{-1})	$5.37x10^{3}$
Sandell's sensitivity coefficient (S) at 283 nm (µg/cm^2/0.001 abs unit)	$7.69x10^{-2}$

Table 9 Evaluation of accuracy and precision of the estimation method of Eletriptan succinate in Phosphate buffered saline (pH=6.4)

Added (µg/ml)	Found (µg/ml) ± SD[a]	Coefficient of variation (CV)	% Relative mean error	Confidence limits[b]
5	5.10 ± 0.14	2.83	2.0	5.10 ± 0.39
20	20.22 ± 0.63	3.13	1.052	20.22 ± 1.60
40	40.18 ± 0.17	0.48	0.386	40.18 ± 0.425
75	75.48 ± 0.47	0.67	0.576	75.48 ± 1.154

$a^n=3^b$ Confidence limits at P=0.95 and two degrees of freedom

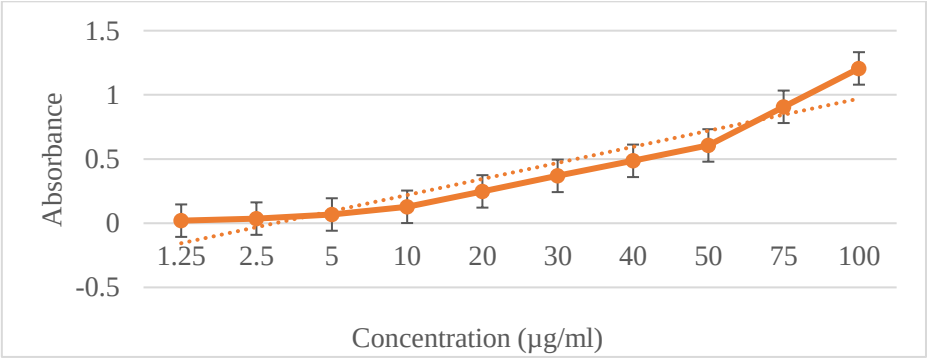

Figure 5 Calibration curve of eletriptan succinate in PBS (pH 6.4)

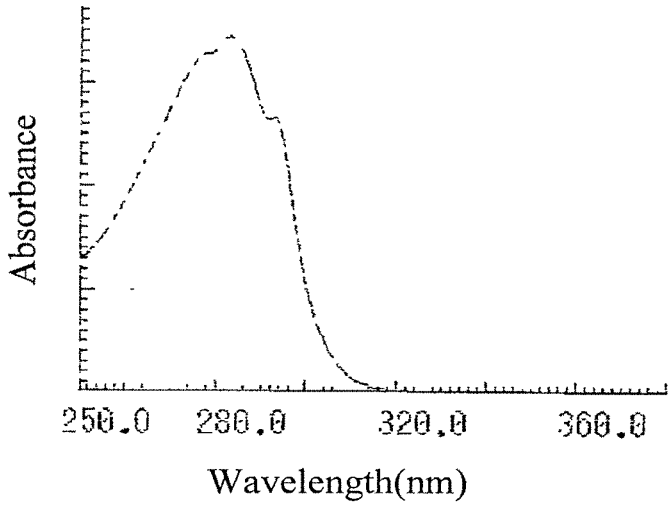

Figure 6 Absorptivity scan of eletriptan succinate in PBS (pH 6.4)

Table 10 Mean absorbance values, regressed values and statistical data of the calibration curve for estimation of Eletriptan succinate in Kreb's solution

Concentration (µg/ml)	Mean Absorbance ± SEM*	Regressed Values
1.25	0.027 ± 0.002	0.022
2.5	0.037 ± 0.001	0.036
5	0.068 ± 0.003	0.067
10	0.137 ± 0.003	0.128
20	0.243 ± 0.003	0.247
30	0.354 ± 0.004	0.368
40	0.475 ± 0.005	0.486
50	0.587 ± 0.004	0.606
75	0.912 ± 0.015	0.908
100	1.214 ± 0.042	1.206

Regression equation Y=0.012X + 0 0057, Correlation coefficient = 0 9994 *Mean of six values

Table 11 Optical Characteristics for Eletriptan succinate in Kreb's solution

Optical properties	Value
Absorption maxima	283
Beer's law limit at 283 nm (µg/ml)	1.25-100
Apparent molar absorptivity at 283 nm (1 mol^{-1} cm^{-1})	$5.72x10^{3}$
Sandell's sensitivity coefficient (S) at 283 nm (µg/cm^2/0.001 abs unit)	$7.23x10^{-2}$

Table 12 Evaluation of accuracy and precision of the estimation method of Eletriptan succinate in Kreb's solution

Added (µg/ml)	Found (µg/ml) ± SD[a]	Coefficient of variation (CV)	% Relative mean error	Confidence limits[b]
5	5.11 ± 0.09	1.65	2.168	5.11 ± 0.209
20	20.22 ± 0.33	1.68	1.095	20.22 ± 0.837
40	39.18 ± 1.17	2.88	2.026	39.18 ± 2.844
75	74.18 ± 1.27	1.77	1.229	74.18 ± 3.214

$a^n=3^b$ Confidence limits at P=0.95 and two degrees of freedom

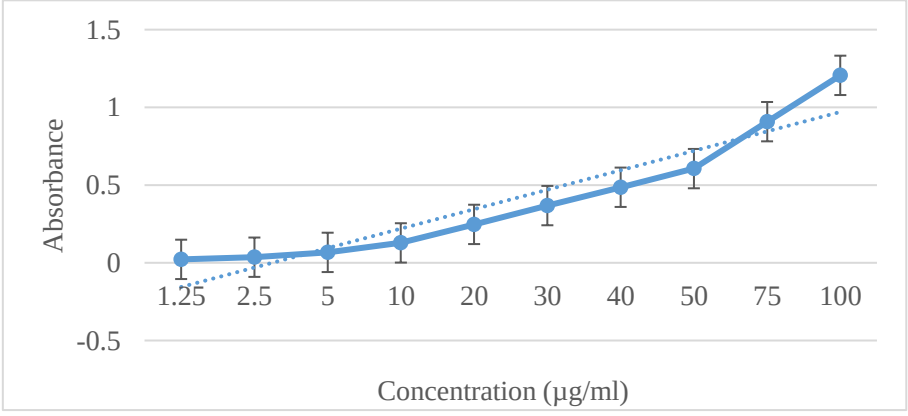

Figure 7 Calibration curve of eletriptan succinate in Kreb's solution

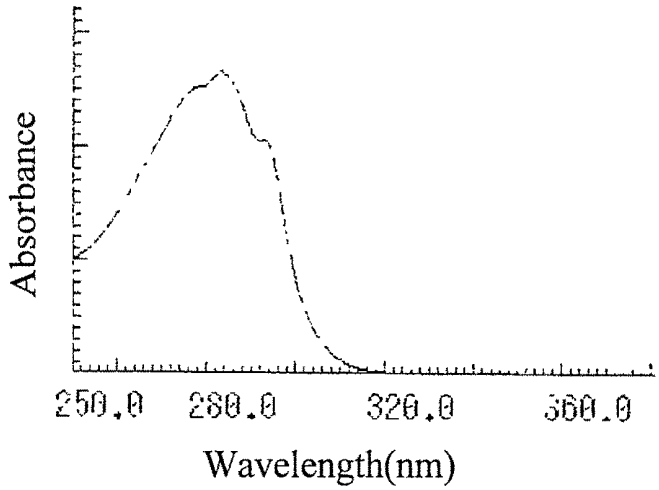

Figure 8 Absorptivity scan of eletriptan succinate in Kreb's solution

Table 13 Mean absorbance values, regressed values, and statistical data from the calibration curve for Eletriptan succinate estimation in Sodium free Kreb's solution.

Concentration (µg/ml)	Mean Absorbance ± SEM*	Regressed Values
1.25	0.023 ± 0.001	0.018
2.5	0.038 ± 0.002	0.032
5	0.061 ± 0.002	0.057
10	0.123 ± 0.002	0.118
20	0.223 ± 0.003	0.227
30	0.324 ± 0.002	0.338
40	0.455 ± 0.003	0.452
50	0.559 ± 0.004	0.562
75	0.860 ± 0.005	0.844
100	1.124 ± 0.012	1.124

Regression equation Y==0,0112X + 0.0031, Correlation coefficient = 0.9996 *Mean of six values

Table 14 Optical Characteristics for Eletriptan succinate in Sodium free Kreb's solution

Optical properties	Value
Absorption maxima	283
Beer's law limit at 283 nm (µg/ml)	1.25-100
Apparent molar absorptivity at 283 nm (1 mol^{-1} cm^{-1})	$4.95x10^{3}$
Sandell's sensitivity coefficient (S) at 283 nm (µg/cm^2/0.001 abs unit)	$8.36x10^{-2}$

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Table 15 Evaluation of the accuracy and precision of the Eletriptan succinate estimation technique in Sodium free Kreb's solution.

Added (µg/ml)	Found (µg/ml) ± SD[a]	Coefficient of variation (CV)	% Relative mean error	Confidence limits[b]
5	5.06 ± 0.10	2.05	1.016	5.06 ± 0.259
20	20.07 ± 0.36	1.82	0.295	20.07 ± 0.906
40	40.18 ± 0.34	0.82	0.276	40.18 ± 0.784
75	76.38 ± 0.49	0.67	1.779	76.38 ± 1.169

a[n]=3[b] Confidence limits at P=0.95 and two degrees of freedom

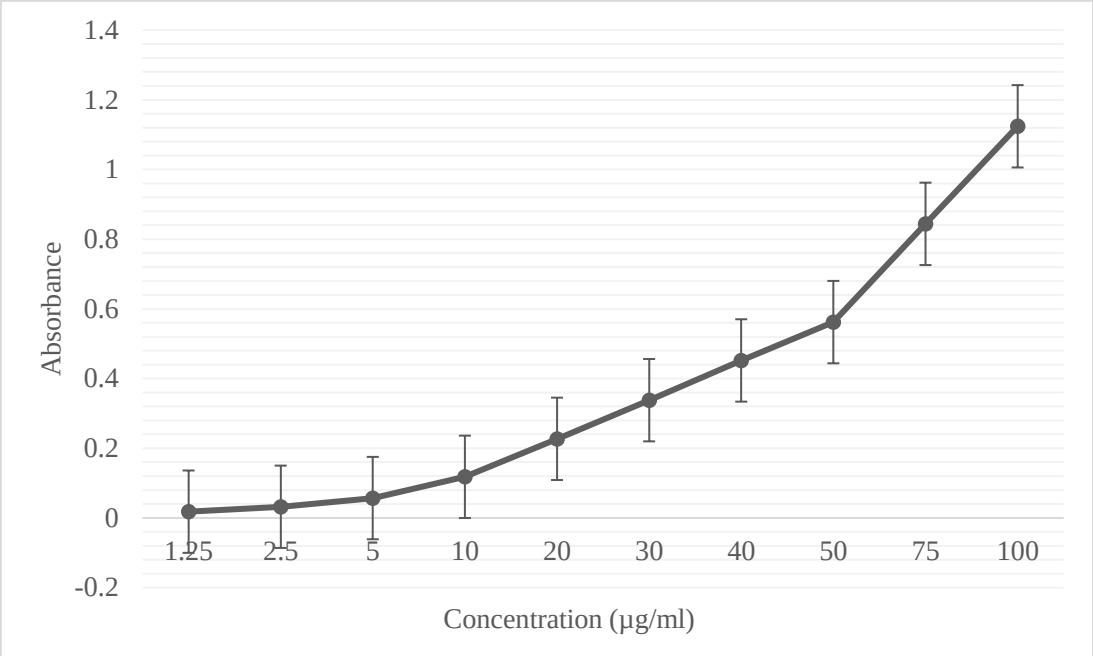

Figure 9 Calibration curve of eletriptan succinate in sodium free Kreb's solution

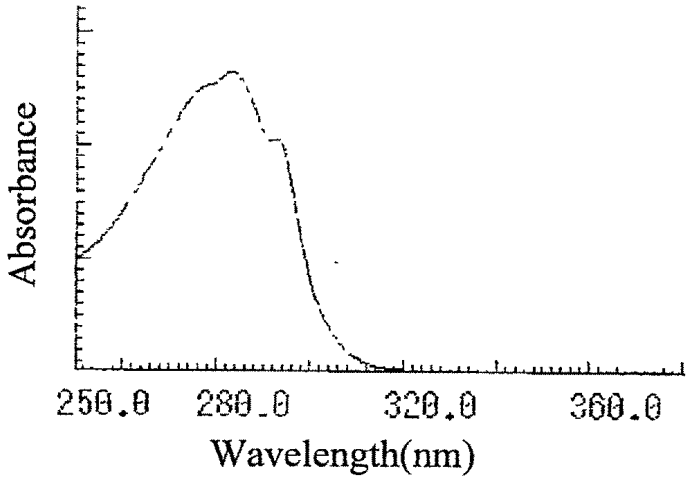

Figure 10 Eletriptan succinate absorption scan in sodium-free Kreb's solution

Table 16 The calibration curve's mean absorbance values, regressed values, and statistical data for estimating Eletriptan succinate in Calcium free Kreb's solution.

Concentration (µg/ml)	Mean Absorbance ± SEM*	Regressed Values
1.25	0.029 ± 0.003	0.021
2.5	0.043 ± 0.003	0.037
5	0.061 ± 0.003	0.068
10	0.139 ± 0.004	0.130
20	0.240 ± 0.005	0.254
30	0.365 ± 0.004	0.378
40	0.500 ± 0.005	0.502
50	0.644 ± 0.009	0.626
75	0.935 ± 0.009	0.936
100	1.251 ± 0.012	1.246

Regression equation Y=0.0124X + 0.0045, Correlation coefficient = 0 9994 * Mean of six values

Table 17 Eletriptan succinate Optical Properties in Calcium-Free Kreb's Solution

Optical properties	Value
Absorption maxima	283
Beer's law limit at 283 nm (µg/ml)	1.25-100
Apparent molar absorptivity at 283 nm (1 mol^{-1} cm^{-1})	$6.69x10^{3}$
Sandell's sensitivity coefficient (S) at 283 nm (µg/cm^2/0.001 abs unit)	$7.27\ x10^{-2}$

Table 18 Evaluation of the accuracy and precision of the Eletriptan succinate estimation technique in Calcium free Kreb's solution

Added (µg/ml)	Found (µg/ml) ± SD[a]	Coefficient of variation (CV)	% Relative mean error	Confidence limits[b]
5	5.08 ± 0.10	1.85	1.345	5.08 ± 0.232
20	19.62 ± 0.34	1.67	1.950	19.62 ± 0.811
40	39.28 ± 0.56	1.41	1.849	39.28 ± 1.362
75	75.53 ± 0.70	0.92	0.700	75.53 ± 1.715

$a^n=3^b$ Confidence limits at P=0.95 and two degrees of freedom

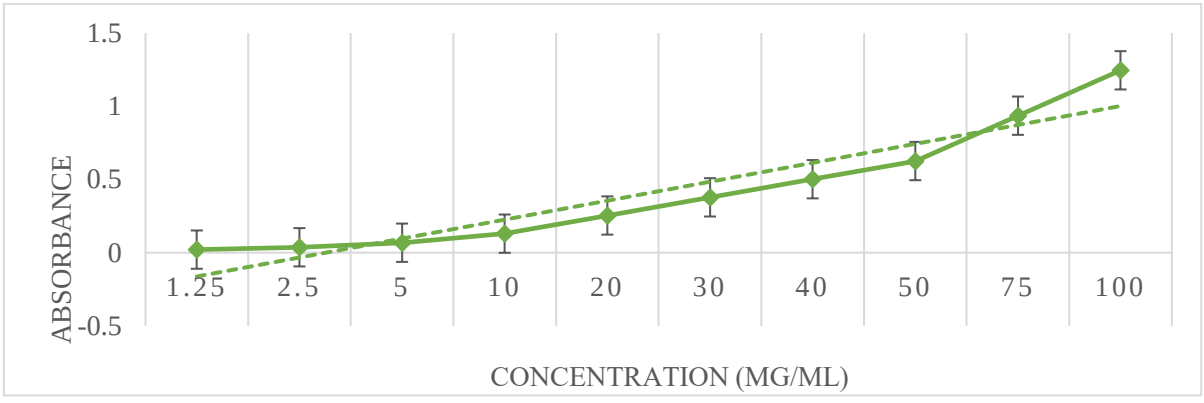

Figure 11 Calibration curve of eletriptan succinate in calcium free Kreb's solution

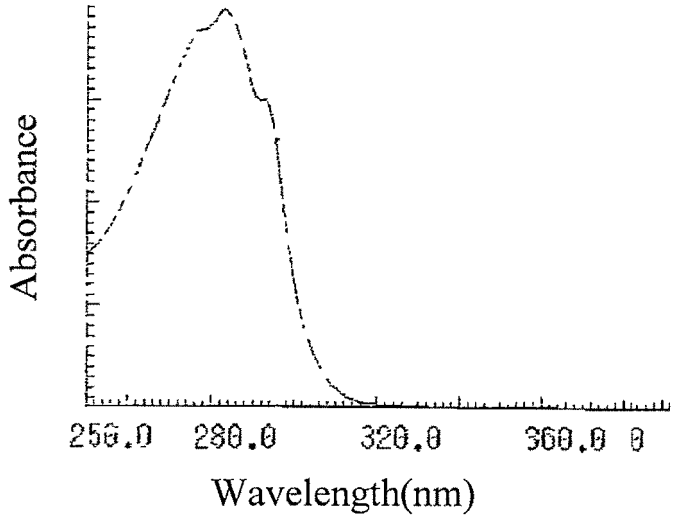

Figure 12 Absorptivity scan of eletriptan succinate in calcium free Kreb's solution

Results And Discussion

When scanned in the UV-Visibie wavelength range of 200-400nm, eletriptan succinate in 0.1N HC1, purified water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution provides distinctive curves. Because the absorptivities at 283 nm were determined to be adequate, this wavelength was chosen as the analytical wavelength and utilized for subsequent experiments.

The strong correlation coefficients in the mentioned above solvents (Tables 3.1, 3.4, 3.7, 3 10, 3.13, and 3.16) demonstrated that drug absorbance as well as concentration were linearly associated. Beer's rule was determined to be respected in the 2.5-100 µg/ml range (Tables 3.2 and 3.3). 1.25-l00µg/ml (Table 3.8, 3.11, 3.14, and 3.17) in phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution. The regression study was performed on the data collected for the experiment, and the experimental data, together with the regressed values, are provided in Tables 3.1, 3.4, 3.7, 3.10, 3.13, and 3.16. The slopes of the regressed lines show that the approaches are moderately sensitive, as indicated by the values of Sandell's sensitivity coefficients.

The low variance of the response variable, S2y,x, for eletriptan succinate in 0.1N HC1 (2.33 x 10'5), purified water (5.29 x 10^{-5}), phosphate buffered saline (pH 6.4) (6.87 x 10^{-5}), Kreb's solution (1.8 x 10^{-4}), sodium free Kreb's solution (1.43 x 10^{-5}) The calcium-free Kreb's solution (2.9 x 10-6) indicates a satisfactory match between the measured and computed data. The minimal variability of the tests is corroborated by the low standard error readings for the solutions utilized to create calibration curves. The slope S^2b variance for eletriptan succinate in 0 IN HC1 (8.6 x 10^{-8}), purified water (1.88 x 10^{-7}), and phosphate buffer saline (pH 6.4) (1.95 x 10^{-8}) Kreb's solution (6.37 x 10'7), sodium free Kreb's solution (5.02 x 10'8), and calcium free Kreb's solution (3.93 x 10'8) suggest that the approach is very sensitive. The compound's high molar absorptivities and low Sandell's sensitivity coefficient values (Tables 3.2,3.5,3.8, 3.11, 3.14, and 3.17) attest to this. In 0.1N HC1, purified water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution, the variance of the intercepts, S2a, was determined as 2.8 x 10'5, 4.09 x 10_s, 6.82 x 10_s, 1.02 x 104, 9.88 x 10'5 and 1.35 x 10'5 correspondingly. The intercept was tested to One-way ANOVA for significance and was not substantially different from zero at 6 degrees of freedom, at P=0.95, indicating that the blank did not interfere with the absorbance readings.

Over a 72-hour time frame, the stability of the medicine was determined in 0.1N HC1, purified water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution. Mean evaluation of variance (ANOVA) The absorbance values of varied volume solutions at

different points in time indicated no significant variation between the results. As a result, it is concluded that eletriptan succinate is stable in the aforesaid solvents for the defined duration.
Eletriptan succinate was estimated in the presence of additional formulation elements such as chitosan glutamate, carbopol 934P, pluronic F-127, and others at acceptable quantities that were present in the final formulations. Neither of the Materials interfered with the estimate of eletriptan succinate using the procedures described above.
The findings of a triple recovery investigation in 0.1N HC1, purified water, phosphate buffer saline (pH 6.4), Kreb's solution, sodium free Kreb's solution, and calcium free Kreb's solution are presented in Tables 3.3, 3.6, 3.9, 3.12,3.15 and 3.18, accordingly, were used to determine the approaches' accuracy and precision. limited relative mean error (%) values suggest limited variability among data points. The precision of the approaches was determined using standard deviation, coefficient of variation (%), and confidence limits. The modest coefficient of variation (%) and confidence bounds show that the approaches are precise.

Conclusion: - Detailed the creation and validation of analytical methodologies for estimating eletriptan succinate in drug-loaded formulations, as well as the assessment of the drug's in vitro permeation mechanism and an in vitro permeation analysis of diverse drugs. formulations over nasal mucosa. The created analytical approach was statistically analysed, and the required statistical parameters were established. The procedures were tested for precision and accuracy. The findings demonstrated that the analytical procedures were selective, accurate, and precise. The approaches may also estimate the eletriptan succinate in the presence of other formulation ingredients. A simple and sensitive high performance liquid chromatography technique with a UV visible detector was devised and validated for estimating eletriptan succinate in rat plasma, CSF (cerebro spinal fluid), and brain tissues. Specificity, robustness, absolute recovery, linearity, sensitivity, precision, and accuracy were all confirmed for the analytical technique. The approach was quick, easy, selective, sensitive, durable, repeatable, accurate, and exact. This approach provides an alternate way for analysing eletriptan in plasma samples as well as a unique method for determining eletriptan in brain homogenate and CSF samples. s. This approach is affordable, simple, and fast, and it may replace other analytical methods for plasma estimates that are sophisticated and need apparatus that is not readily available in all laboratories. further pricey Whereas the approach proposed for estimating eletriptan succinate in CSF and brain tissue is innovative, as no estimation method has previously been described. In addition, an analytical technique for estimating residual glutaraldehyde in chitosan glutamate microspheres was devised and confirmed.

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