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METHOD VALIDATION OF PERAMPANEL BY A SIMPLE UV SPECTROPHOTOMETRIC METHOD FOR IMPROVING ITS AQUEOUS SOLUBILITY USING HYDROTOPES

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ABSTRACT
Hydrotropy is a solubilization process whereby addition of a large amount of second solute results in an increase in the aqueous solubility of another solute. Hydrotropic agents are ionic organic salts. Additives or salts that increase the solubility in a given solvent are said to salt in the solute and those salts that decrease the solubility are said to "salt out" the solute. Perampanel is an orally active, non-competitive, and selective alpha- amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid glutamate receptor antagonist, with anti-epileptic activity. In UV spectroscopy the scan range was set between 200-400nm to obtain the maximum absorption by the compound. The wavelength at which Perampanel showed maximum absorbance was found to be 286.4nm. The UV absorption spectrum of Perampanel. The developed method has been validated according to ICH guidelines for linearity, range, precision (repeatability), accuracy, robustness, LOD and LOQ parameters. The results showed that the developed method is simple, precise, accurate and robust. Therefore, the method can be applied for routine analysis of Perampanel in bulk and pharmaceutical formulation.

Keywords: Additives, Hydrotropy, ICH, LOD, LOQ.

INTRODUCTION

Hydrotropic solutions do not show colloidal properties and involve a weak interaction between the hydrotropic agent and solute. Hydrotropy designate the increase in solubility in water due to the presence of large amount of additives and its hydrotropic agents shown in table 1 [1, 2].

Mechanism of hydrotropic solubilization

Self-association of hydrotropes forms aggregates. It attracts the solute molecules into the aqueous phase. So as concentration of hydrotropic agent increases, it results in increase in solubility in figure 1 [3].

Advantages of hydrotropic solubilization technique

- It excludes use of organic solvents, thus avoids the problem of residual solvent toxicity.
- It is new, simple, cost effective, safe and environmental friendly method for the analysis of poorly water-soluble drugs.
- It only requires mixing of the drug and the hydrotrope in water.
- Hydrotropy is superior in comparison to other solubilization method.
- It does not require chemical modification of lipophilic drugs [4, 5].

UV- Spectroscopy

UV spectroscopy is the absorption or reflectance spectroscopy of the ultraviolet and adjacent visible regions of the electromagnetic spectrum that measures the amount of UV that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. It is also known as UV-visible spectrophotometry. Because of its low cost and ease of implementation, this methodology is widely used in a wide range of applied and fundamental applications. The only requirement is that the sample absorb in the or transmitted range, indicating that it is a chromophore.

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The only requirement is that the sample absorb in the or transmitted range, indicating that it is a chromophore. Aside from the wavelength, the parameters of interest are absorbance (A), transmittance (%T), and reflectance (%R), as well as their variations over time and its instrumentation was shown in figure 2 [6-8].

Applications of UV-Vis spectroscopy

- **DNA and RNA analysis**: One common application is quickly determining the purity and concentration of RNA and DNA [9].
- **In Pharmaceutical Industry**: Processing UV-Vis spectra using mathematical derivatives allows overlapping absorbance peaks in the original spectra to be resolved to identify individual pharmaceutical compounds for example, benzocaine, a local anesthetic, and chlortetracycline [10].
- **Bacterial Culture**: UV-Vis spectroscopy is often used in bacterial culturing. OD measurements are routinely and quickly taken using a wavelength of 600 nm to estimatethe cell concentration and to track growth.600 nm is commonly used and preferred due to the optical properties of bacterial culture media in which they are grown and to avoiddamaging the cells in cases where they are required for continued experimentation.
- **Beverage Analysis**: Another common application of UV-Vis spectroscopy is the identification of specific compounds in beverages. Caffeine content must be within certain legal limits, which UV light can help to determine. Certain classes of coloured substances, such as anthocyanin found in blueberries, raspberries, blackberries, and cherries, are easily identified in wine for quality control using UV-Vis absorbance by matching their known peak absorbance wavelengths [11-14].

MATERIALS AND METHODS

Drug Profile

Perampanel is an orally active, non-competitive, and selective alpha- amino-3-hydroxy-5- methyl-4-isoxazolepropionic acid (AMPA) glutamate receptor antagonist, with anti-epileptic activity. AMPA receptors play a key role in mediating the action of glutamate at the excitatory synapse. Preclinical research showed the AMPA receptor blockade to constitute a promising target for antiepileptic drug therapy [15].

METHOD DEVELOPMENT

Preliminary solubility study (Selection of hydrotropic agent)

3mg of drug was added to a screw capped 25 ml of volumetric flask containing different aqueous systems viz. distilled water, different combination of hydrotropic agent. The volumetricflasks were shaken mechanically for 12 hrs. at 25±1°C in a mechanical shaker. These solutions were allowed to equilibrate for next 24 hrs and centrifuged. The supernatant liquid was taken for appropriate dilution after filtered through Whatman filter paper no.41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in the solubility of Perampanel was found to be 15 times more in 10M urea as compared to solubility studies in other solvents [16].

Estimation of Perampanel by UV Spectroscopy

Preparation of standard stock solution

100mg of Perampanel weighed and transferred to 100mL volumetric flask, dissolved and made up the volume with distilled water. Stirred in magnetic stirrer for 3 hours.

Preparation of Test solution

For the estimation of drugs in the commercial formulation, 25 tablets were weighed. Their average weight was determined and was grounded to fine powder using a glass mortar and pestle. Tablet powder equivalent to about 100mg of Perampanel was transferred to 100ml volumetric flask, volume was made up to the mark with distilled water and stirred in magnetic stirrer for 3 hours. The resulting solution was mixed and filtered using filter paper. This solution was used for further analysis [17].

Selection of wavelength range for estimation

Selection of analytical wavelength was done by scanning above solution in the range 200 - 400nm. The wavelength at which Perampanel showed maximum absorbance was found to be286.4nm.

Validation

The developed method was validated as per ICH guidelines. The parameters assessed were Linearity, Accuracy, Precision (repeatability), LOD, LOQ and Robustness.

Linearity

The aliquots of concentration ranging from 5-50 g/ml were prepared in triplicate, but linearity was found to be between 5-40 g/ml concentrations. The solutions were analyzed at wavelength 286.4nm and absorbances were noted. Calibration curve of Perampanel was constructed by plotting the absorbance v/s concentration of Perampanel. The correlation coefficient (r^2) of least square linear regression for Perampanel was calculated [18].

Accuracy

The accuracy was determined by calculating % recovery of Perampanel. It was carried out by adding known amounts of analyte corresponding to 3 concentration levels 80, 100 and 120% and results were expressed as % recovery.

Precision

The precision of analytical method was studied by performing intermediate precision and repeatability (intraday precision). Intermediate precision of the method was evaluated on 3 consecutive days by carrying out the same procedure. Repeatability studies were carried out by estimating responses of working standard solution (concentration of Perampanel: 10µg/ml) for 3 times. The results were reported in terms of percentage relative standard deviation (%RSD) [19].

Robustness

By deliberate change in wavelength i.e., 281.4nm and 291.4nm of concentration 10 mcg of Perampanel was analyzed, in the same environmental condition [20].

Limit of detection (LOD)

LOD is the lowest amount of analyte in sample that can be easily detected but not necessarily quantified. LOD was calculated by following formula:

$$LOD = 3.3 \times \sigma / S$$

Where,

σ = standard deviation and,
S = slope of the regression coefficient

Limit of quantification (LOQ)

LOQ is the lowest amount of analyte in sample that can be easily detected and quantified with suitable precision and accuracy was calculated by following formula: [21]

$$LOQ = 10 \times \sigma / S$$

Where,
σ = standard deviation and,
S = slope of the regression coefficient.
Absorption spectrum shows λ_{max} of Perampanel at 286.4nm.

RESULT AND DISCUSSION

Linearity

The absorbance is proportional to the concentration and linear in range of 5-40µg/ml (Tab:6). The value of r^2 was 0.9977 which is well within acceptance limit ($r^2 < 1$).

Table 1: Classification of hydrotropic agents:

Type	Example
AromaticAnionics	Sodium Benzoate Sodium Salicylate Sodium Cinnamate
AromaticCationics	Para aminobenzoic AcidHydrochloride Procaine Hydrochloride Caffeine
Aliphatic And LinearCompounds	Sodium AlkanoateUrea N, N-dimethyl Urea

Table 2: Description of drug.

Name	**Perampanel**
Category	Anticonvulsants
Mechanism of action	Inhibits AMPA glutamate receptor
IUPAC	5'-(2-cyanophenyl)-1'-phenyl-2,3'-bipyridinyl-6'(1H)-one
Molecular formula	$C_{23}H_{15}N_3O$
Molecular weight	349.4g/mol
Melting point	175–176 °C
Solubility	Freely soluble; N-methyl pyrrolidone Slightly soluble; methanol and ethanol Poorly soluble; heptane and water.[11]

Table 3: Chemicals and reagents used

Chemical and reagents	**Manufacturer**
Distilled water	Sadbhavna chemicals
Urea	Isochem laboratories

Table 4: Instruments used

Instruments	**Manufacturer**
Digital balance	Notebook series digital scale

Analytical balance	Shimadzu corporation
Magnetic stirrer	Rotek instruments
UV-Vis Double Beam Spectrophotometer	Systronics

Table 5: Materials used

Drug	Manufacturer
Perampanel	Gift sample from Manus AkttevaBiopharma LLP, Ahmadabad
Perampanel 4mg	Health clinic Mogral

Table 6: Linearity of Perampanel

Concentration (µg/ml)	Absorbance
5	0.230
10	0.456
15	0.684
20	0.918
25	1.142
30	1.372
35	1.512
40	1.701

Table 7: Accuracy results of Perampanel

Reps	Conc (%)	Conc. of sample (µg/ml)	Conc.ofstd. added (µg/ml)	Absorbance	% recovery	Mean % recovery	% RSD
1	80	10	8	0.809	99.50	99.41	0.14
2		10	8	0.808	99.25		
3		10	8	0.809	99.50		
1	100	12	12	1.052	97.52	97.65	0.12
2		12	12	1.053	97.72		
3		12	12	1.503	97.72		
1	120	15	18	1.426	97.24	97.36	0.07
2		15	18	1.427	97.37		
3		15	18	1.426	97.24		

Table 8: Intraday precision (Repeatability) results of Perampanel

Time(hour)	Conc (µg/ml)	Absorbance	Average	SD	% RSD
0	10	0.456	0.461	0.00458	0.993
1		0.462			
2		0.465			
0	20	0.918	0.922	0.00529	0.5739
1		0.928			
2		0.920			
0	30	1.372	1.373	0.001	0.0728
1		1.374			
2		1.373			

Table 9: Intermediate Precision Results of Perampanel

Day	Conc (µg/ml)	Absorbance	Average	SD	% RSD
1	10	0.461	0.462	0.0036	0.78
2		0.459			
3		0.461			
1		0.684			

2	15	0.682	0.68	0.0012	0.17
3		0.684			
1	20	0.918	0.919	0.0011	0.1256
2		0.918			
3		0.920			

Table 10: Robustness results of Perampanel

Reps	Wavelength (nm)	Conc (µg/ml)	Absorbance	Average	SD	% RSD
1	281.4	10	0.446	0.446	0.0012	0.25
2			0.448			
3			0.446			
1	286.4	10	0.456	0.456	0.001	0.21
2			0.457			
3			0.455			
1	291.4	10	0.451	0.452	0.0015	0.26
2			0.453			
3			0.453			

Table 11: Summary of validation parameters.

PARAMETERS	VALUES
Max. Wavelength	286.4 nm
Beers law (µg/ml)	5-40
Regression equation	Y=0.0436X+0.0376
Slope	0.0376
Intercept	0.043
Regression Coefficient (R^2)	0.99772
Repeatability (n=3) % RSD	0.993
LOD (µg/ml)	5.93 µg/ml
LOQ (µg/ml)	17.92µg/ml

Figure 1: Mechanism of hydrotropic solubilization.

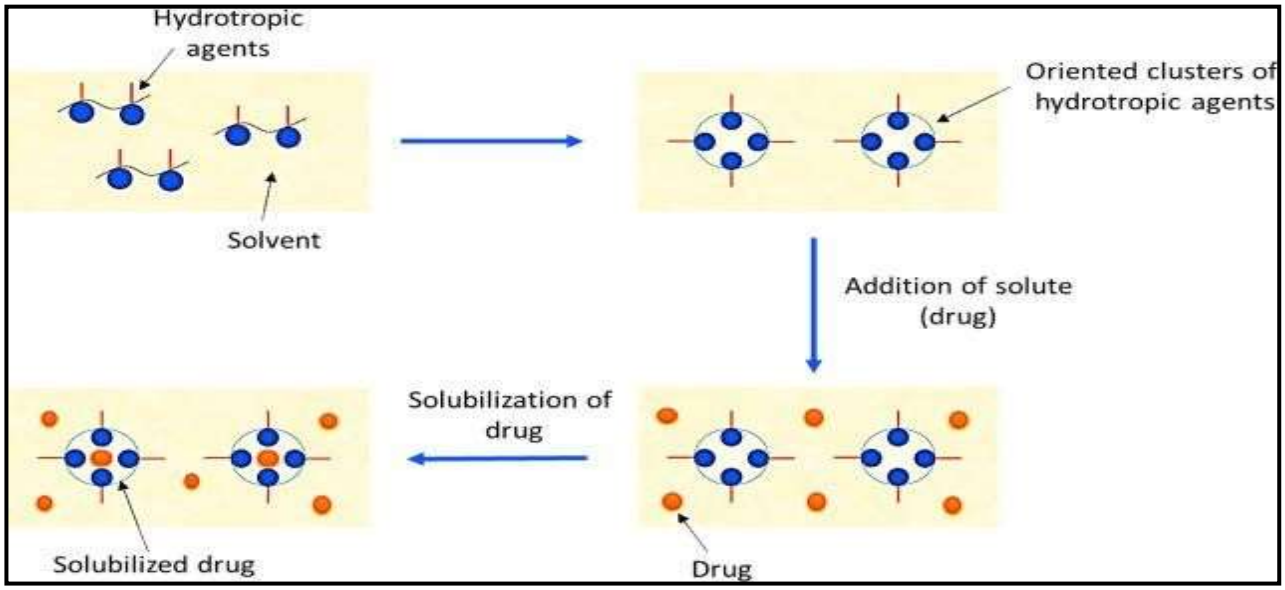


Figure 2: Schematic diagram of UV-Vis double beam Spectrophotometer.

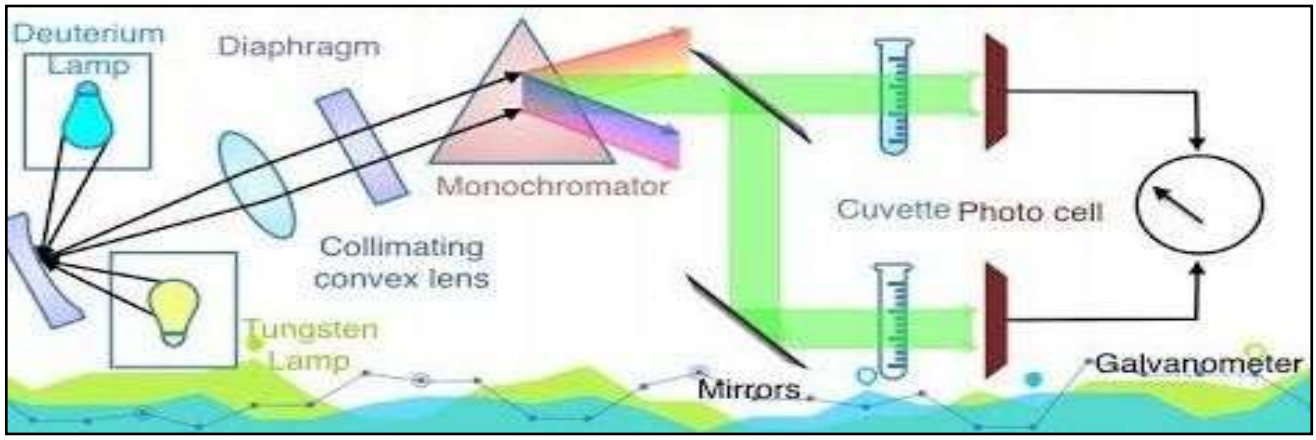


Figure 3: Chemical structure of Perampanel

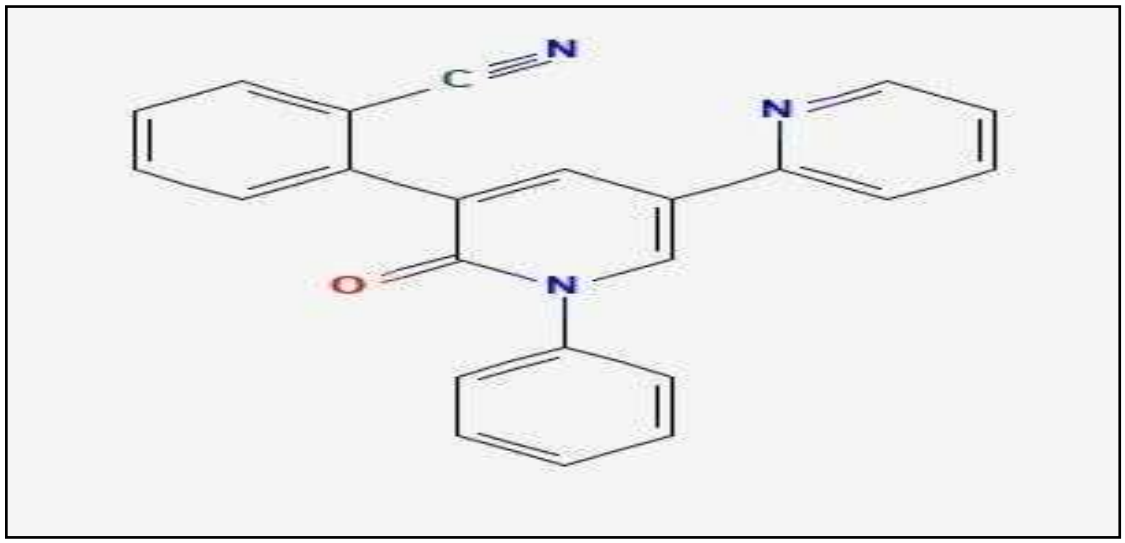


Figure 4: UV spectra of Perampanel

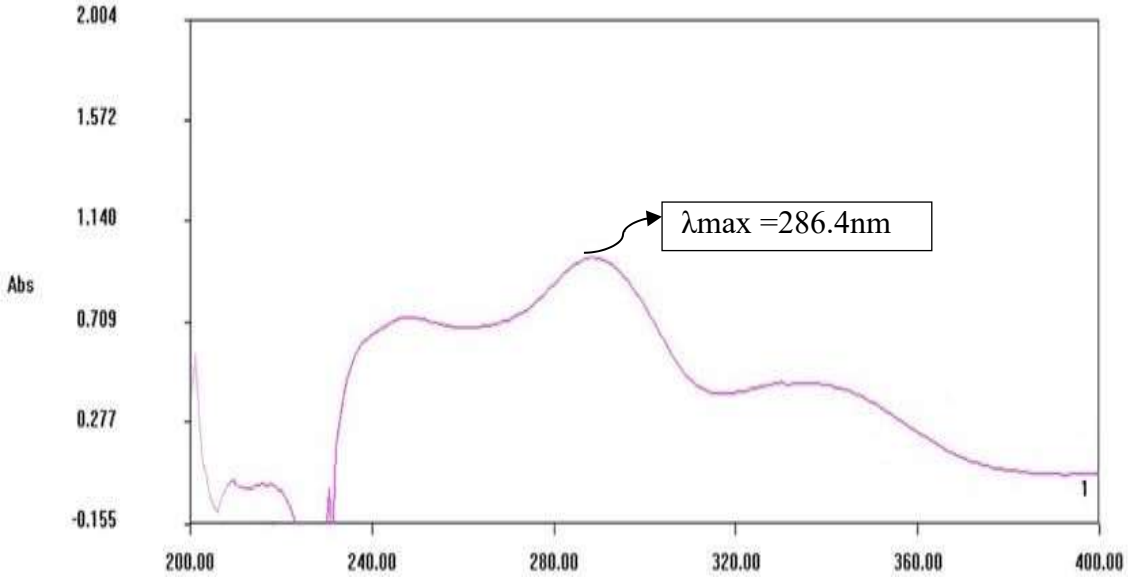


Figure 5: UV spectra of urea

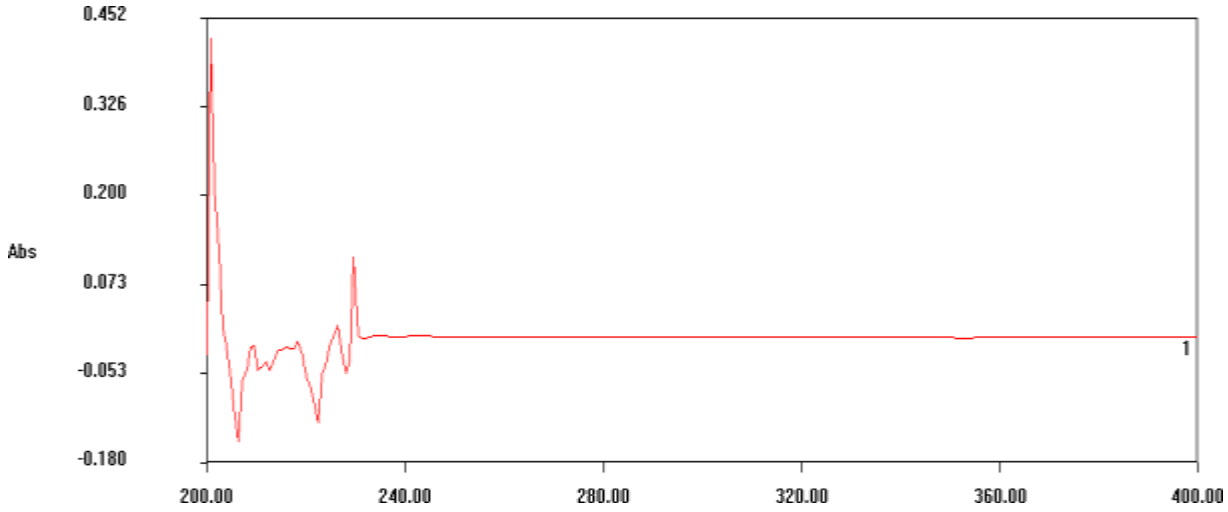


Figure 6: UV spectra of different conc. of standard.

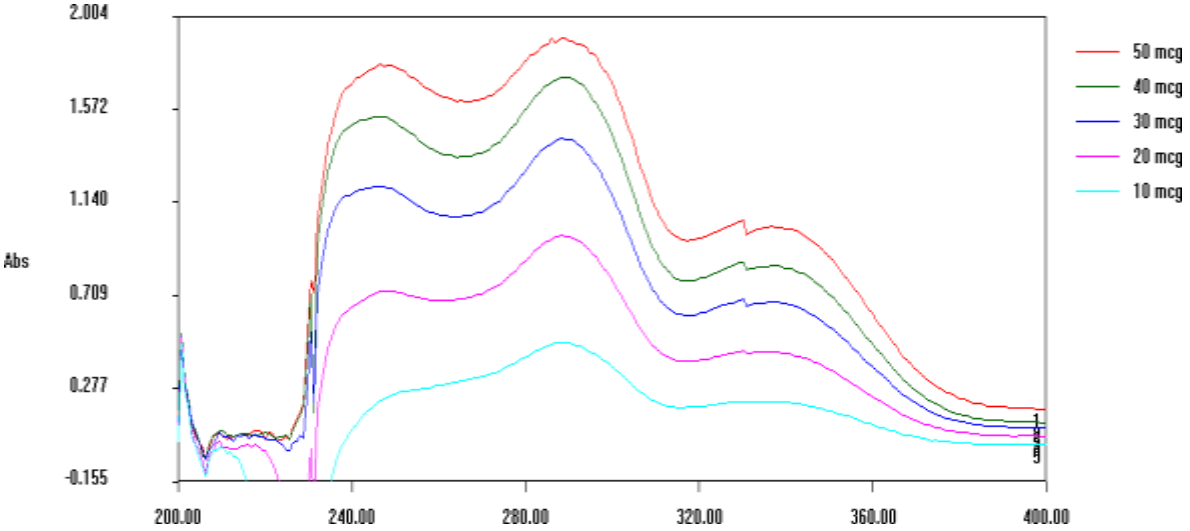


Figure 7: Linearity curve for Perampanel

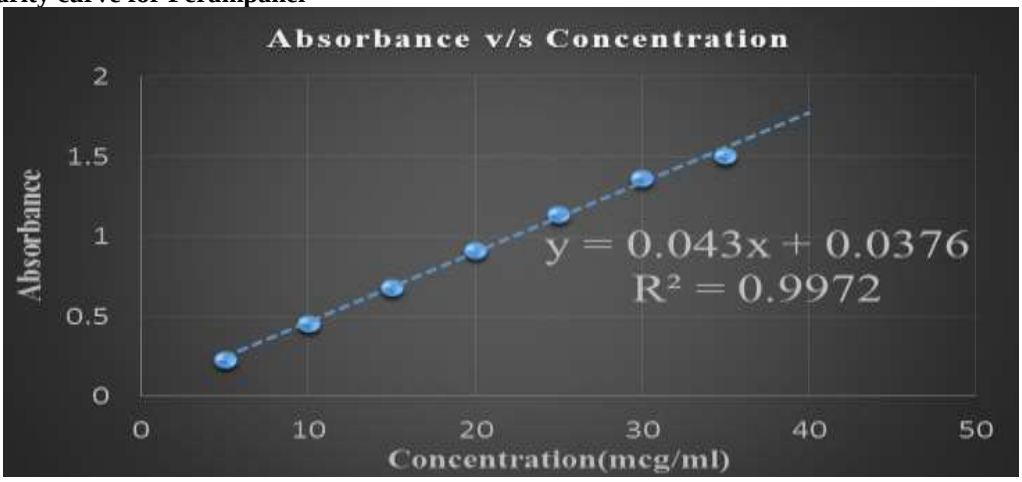
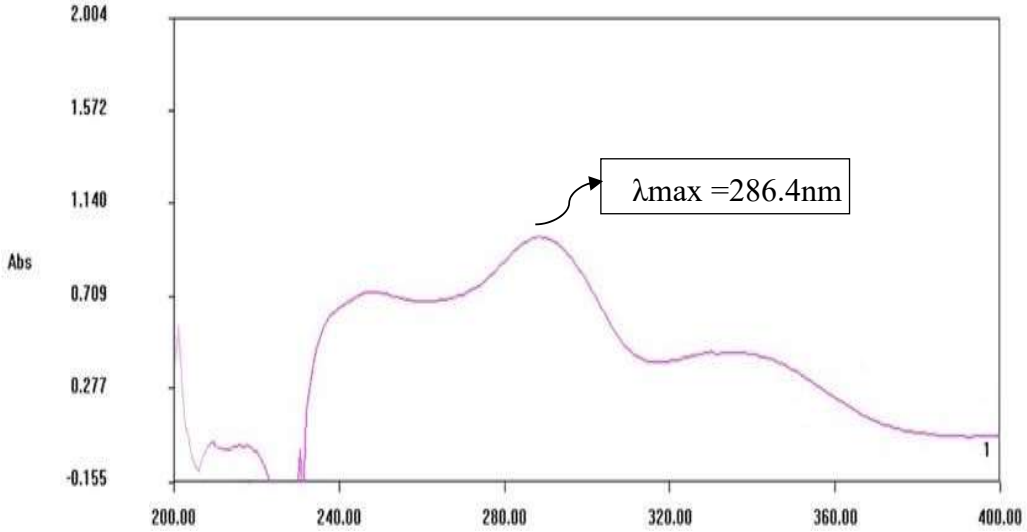


Figure 8: UV spectra of Perampanel.



Accuracy

The percentage recoveries of the results indicate that the recoveries are well within acceptance range (%RSD < 2), therefore the method is accurate.

Precision

The % RSD < 2 values obtained shows that method developed is precise.

Robustness

The deliberate change in wavelength, in the same environmental condition, gave there liable results. (Table 10)

The hydrotropic solubilization method was found to be simple, accurate, economic and rapid for routine analysis of Perampanel in bulk and pharmaceutical dosage form.10M urea solution improved solubility of Perampanel 15 times than in distilled water. In UV spectroscopy the scan range was set between 200-400nm to obtain the maximum absorption by the compound. The wavelength at which Perampanel showed maximum absorbance was found to be 286.4nm. The UV absorption spectrum of Perampanel is shown in the figure 8. To ascertain the effectiveness of the method various validation parameters were checked.

CONCLUSION

Analytical method development is a tedious process for poorly soluble drug; since solubility is limiting factor in its estimation. Most of the poorly soluble drugs are estimated using organic solvents. Organic solvents are toxic, having high cost and cause error due to volatility. Hydrotropic solubilization can be utilized to solubilize poorly water-soluble drugs.

Here an attempt has been made to develop UV Spectrophotometric method for estimation of Perampanel by hydrotropic solubilization in bulk and pharmaceutical formulation.10M Urea solution was used as hydrotropic agent. The developed method has been validated according to ICH guidelines for linearity, range, precision (repeatability), accuracy, robustness, LOD and LOQ parameters. The results showed that the developed method is simple, precise, accurate and robust. Therefore, the method can be applied for routine analysis of Perampanel in bulk and pharmaceutical formulation.

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