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VALIDATION AND DEVELOPMENT METHOD FOR THE ESTIMATION OF TEMOZOLOMIDE BY UV- SPECTROSCOPY

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ABSTRACT:

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities ,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.

By now it should be quite apparent that pharmaceutical analysts play a major role in assuring the identity, safety, efficacy, and quality of drug product, safety and efficacy studies required that drug substance and drug product meet two critical requirements.

1. Established identity and purity.

Established bio availability/dissolution

KEY WORDS: quality control (QC), identity and purity, Research and development (R&D), availability/dissolution, active pharmaceutical ingredients

INTRODUCTION:

Analytical chemistry

A branch of chemistry that deals with the identification of compounds and mixtures (qualitative analysis) or the determination of the proportions of the constituents (quantitative analysis).The techniques commonly used are titration, precipitation, spectroscopy, chromatography, etc

Analytical chemistry serves the needs of many fields:

- ✓ In industry, analytical chemistry provides the means of testing raw materials and for assuring the quality of finished products whose chemical composition is critical. Many household products, fuels, paints, pharmaceuticals, etc. are analyzed by the procedures developed by analytical chemists before being sold to the consumer.
- ✓ The nutritional value of food is determined by chemical analysis for major components such as protein and carbohydrates and trace components such as vitamins and minerals. Indeed even the calories in a food are often calculated from its chemical analysis.
- ✓ In medicine, analytical chemistry is the basis for clinical laboratory tests, which help physicians to diagnose disease and chart the progress in recovery.
- ✓ Environmental quality is often evaluated by testing for suspected contaminants using the techniques of analytical chemistry.
- ✓ Analytical chemists also make important contributions to fields as diverse as forensic chemistry, archaeology, and space science[1].

Chromatography:

Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time."

A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it.

Chromatograph separates the chemical mixture either liquid or gas into its components by differential distributions of the solutes, as they flow with different rate over the stationary phase. Type of the technique used for the separation of complex mixtures depends on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species[2].

Detectors

The sensitivity of universal detector for HPLC has not been devised yet. Thus it is necessary to select a detector on the basis of the problem.

UV visible photometers and spectrometers

Optical detectors based on UV -visible absorption are the workhorses of HPLC, constituting over 70% of the all detection systems in use. Basically three types of absorbance detectors are available: a fixed wavelength detector, a variable wavelength detector, and a scanning wavelength.

Fixed Wavelength Detectors

A fixed wavelength detector uses a light source that emits maximum light intensity at one or several discrete wavelengths that are isolated by appropriate filters.

Variable Wavelength Detector

This is relatively wide band pass it offers a wide range of selection of UV and visible wavelengths but it is costly one compared to fixed wavelength detectors.

Photo Diode Array (PDA) Detector

To obtain a real time spectrum for each solute as it elutes, solid-state diode arrays are required. The diode arrays work in parallel simultaneously monitoring all wavelengths.

Digital electronic integrators are widely used today in HPLC for measuring peak areas. These devices automatically sense peaks and print out the areas in numerical forms. Computing integrators are even more sophisticated and offer a number of features in addition to basic digital integration because these devices have both memory and computing capabilities to upgrade integrating parameters to maintain accuracy as the separation progress and eluting peaks become broader. Many of these devices print out a complete report including names of the compounds, retention times, peak areas and area correction factors. With the help of

peak area and height values, the peak width can be calculated (considering the peak as a triangle) and it can also be used for the calculation of number of theoretical plates[10].

Introduction to UV/Visible Spectrophotometers

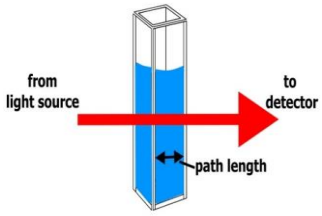
UV/Visible spectrophotometry is a mature and established technique, with inbuilt flexibility to detect and measure millions of compounds (analytes) in a wide variety of sample matrices. This technique is used within a wide variety of analytical chemistry laboratories, such as within the following sectors: -

- Life Science commercial enterprises.
- Research and Teaching.
- University, Life sciences, Chemistry.
- Hospitals and Clinics.
- Food and Drinks manufacturing.
- Environmental.
- Water Suppliers.
- Forensics.
- Pathology.
- Pharmaceuticals.
- Nutraceuticals.

Theory:

Some molecules can absorb certain ideal wavelengths of ultra-violet (UV) or visible light. This means that compounds containing these molecules have a UV/Visible chromophore. At those given wavelengths, the amount of UV/Visible chromophore in a sample may be measured. Generally, the higher the amount of UV/Visible chromophore detected, the higher the concentration of that compound.

Light generated by certain lamps, such as tungsten, deuterium or xenon, is separated into discrete bands of wavelengths of light by a monochromator, and then is passed through a sample via a slit. A sample with a UV/Visible chromophore absorbs a certain amount of light, the remaining light is detected by a detector. Liquid samples are held in cuvettes (or cells) within a spectrophotometer. The width of the cell, in which light passes through the sample is the pathlength, a standard pathlength used by many users is 10 mm.



The Beer-Lambert law is then used to determine the concentration of a specific analyte (compound) in a clear liquid sample at a specific wavelength.

A = Є x l x c

Where, at a specific wavelength,

A is the measured **Absorbance**,

Є is the molar absorbtivity or extinction coefficient (M-1 cm-1),

l is the pathlength (cm),

c is the analyte concentration (M).

The Beer-Lambert law provides for a linear relationship, however, there are some restrictions to the law, and the linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Provided that the concentration is measured in the linear part of the calibration curve for each specific analyte, the law will apply in practice.

If Transmittance measurements are required, then there is the following relationship: -

A = -log T

Cuvettes are made of thin, uniform glass or plastic that is highly transparent to light at the wavelengths used. Some wavelengths such as those in the ultraviolet region (190 to 300 nm) require cuvettes made of other materials such as quartz or silica. The surfaces of the cuvette through which light passes through should be clean, clear and free of finger prints and grease.

For very low analyte concentrations, cells of pathlength larger than 10 mm may be used. For very high analyte concentrations, cells of pathlength smaller than 10 mm pathlength may be used. Of course, it is possible to dilute solutions which have high analyte concentrations, so that their Absorbances are within the Absorbance range of the spectrophotometer and vice-versa for solutions which have low analyte concentrations.

UV/Visible spectrophotometers are mainly used to measure the Absorbance/Transmittance of analytes within liquids. These liquids are placed inside cuvettes, which are then positioned within the spectrophotometer. These cuvettes and the liquids themselves, have their own Absorbance/Transmittance values.The Absorbance/Transmittance values of the cuvettes and liquids must be accounted for. This is performed using a blank (reference).

In single beam spectrophotometers, a cuvette filled with the liquid, but without analyte, is placed in the spectrophotometer, and at the required wavelength is 'zeroed'. When a cuvette filled with the liquid and the analyte, is subsequently placed in the spectrophotometer, the difference in Absorbance/Transmittance value is therefore attributed to the analyte itself.

In double beam spectrophotometers, there are two beams of light. So initially, two cuvettes filled with the liquid, but without analyte, are placed in each beam the spectrophotometer. At the required wavelength, the spectrophotometer is 'zeroed'. Then a cuvette filled with the liquid and the analyte, is subsequently placed in the sample beam of the spectrophotometer, so that the difference in Absorbance/Transmittance value is therefore attributed to the analyte itself. The advantage here, is that any variations of the liquid, such as slight precipitation, chemical reactions and temperature etc., apply to both the sample and the blank, at the same time.

Wavelength Scanning:

In order to determine the specific wavelength, at which a user wishes to make a measurement, it is prudent to perform a wavelength scan. A wavelength scan is a plot of Absorbance on the y axis and wavelength on the x axis, for a analyte. This specific wavelength is often the Lambda max i.e. the wavelength which gives rise to the highest clearly resolved Absorbance value. The scan itself may also be useful in aiding in the identification of the analyte.

Single Wavelength Quantitative Measurements:

Once a specific wavelength has been chosen, the spectrophotometer is set at that chosen wavelength. A calibration or standard curve of Absorbance or Transmittance versus concentration needs to be plotted, (where various levels of concentration of a known standard and the resultant Absorbance/Transmittance readings are plotted). The amount of the unknown analyte in the sample is read off against the curve, or as a factor is derived from a calibration curve.

In general, Concentration = Factor x Absorbance.

The factor is generally the gradient of a calibration curve and its intercept on the y axis. A factor is specific to each individual spectrophotometer, for a analyte, at a specific wavelength, using a specific blank, after a specific type of sample pre-treatment.

Before a standard or sample reading is taken, a blank/reference (this material should be like the standard/sample but does not contain the analyte of interest) should be inserted in the spectrophotometer and the instrument zeroed for that blank at that the chosen wavelength.

The measured Absorbance/Transmittance of the standards and samples should not be beyond the spectrophotometer's measuring range. Depending on the stray light of the spectrophotometer, the best Absorbance's to aim for are 0.6 to 0.7 Absorbance units.

Stray light is the detected light of any wavelength that is outside the bandwidth of the selected wavelength. The greater the stray light, the higher the degree of inaccuracy of Absorbance readings which are more than around 1.5 Absorbance units.

Single Beam Spectrophotometers:

Here a single beam of light is passed through a single sample container and the resulting light is detected by a detector. In the case of single beam spectrophotometers, most detectors are silicon diodes. A filter and a monochromator with a slit, split the light to the chosen wavelength. The bandwidth (bandpass) of the spectrophotometer, relates to the size of the monochromator' slit for example 4 nm.

A narrow bandwidth will produce wavelength scans of higher resolution than a wider bandwidth. However, there will be less light energy to reach the detector, consequently, there can be a loss of sensitivity at narrow bandwidths. If a filter alone is used to split the light, a very wide bandwidth result. In addition, it is not possible to perform wavelength scanning, as discrete filters need to be physically inserted for each wavelength range

used. Single Beam Spectrophotometers are of the simplest in design hence have lower capital and maintenance prices than other spectrophotometer types.

Double Beam Spectrophotometers:

Here the light leaving the monochromator is split, using a beam splitter, into a sample beam and a reference beam. After each beam of light is passed through its respective sample/reference (blank) container, each beam is then detected by its own detector. The sample and reference are simultaneous /measured/scanned, saving time and providing for optimum accuracy.

Hence the double beam spectrophotometer can have two detectors. Most detectors are silicon diodes. Double beam spectrophotometers ensure that any fluctuations in the light emitted from the lamp are applied equally to both the sample and the reference beams, hence double beam spectrophotometers offer around ten times better stability and 2 to 5 times lower baseline noise, than single beam spectrophotometers of the same brand.

For greater sensitivity, the two silicon diode detectors may be substituted with a single photomultiplier detector. Photomultiplier detectors are used in the more expensive spectrophotometers and have a maximum wavelength range of 900 nm. In practice, most UV/Visible measurements occur within the 190 to 800 nm wavelength range.

End window photomultipliers can collect up to 100 times lighter. Hence it is possible to measure highly light scattering samples. A single photomultiplier detector simultaneously measuring both sample and reference beams, is more precise than a two-detector system. Good accuracy and performance occur, even at narrow opticalbandwidths.

Before a standard or sample reading is taken, a blank/reference (this material should be similar to the standard/sample, but does not contain the analyte of interest) should be inserted in the spectrophotometer and the instrument zeroed for that blank at that the chosen wavelength.

The measured Absorbance/Transmittance of the standards and samples should not be beyond the spectrophotometer's measuring range. Depending on the **stray light** of the spectrophotometer, the best Absorbance's to aim for are 0.6 to 0.7 Absorbance units.

Stray light is the detected light of any wavelength that is outside the bandwidth of the selected wavelength. The greater the stray light, the higher the degree of inaccuracy of Absorbance readings which are more than around 1.5 Absorbance units.

Advantages:

Some commonly required measurements, such as time plots, thermal melts, kinetics, concentrations of oligonucleotides, levels of chemical elements, amounts of proteins, observance of cell growth, Transmittance of light through lenses etc., may then be produced using UV/Visible spectrophotometers.

Common Accessories and Consumables:

Autosamplers: These are useful when large numbers of samples require unattended measurement.

Calibration standards: As part of the instrument validation process, these are used to check the wavelength accuracy, Absorbance accuracy, stray light and bandwidth of spectrophotometers. They can be solid filters or solutions.

Cells (cuvettes): A wide variety of rectangular or circular cells are available. Typical pathlengths range from 1 to 100 mm. Working volumes can start from 1 µL, especially if nano cells are used. Flow cells are used if liquids are to be continuously passed through a single cell. Fibre optic cells may be used if the sample is some distance from the spectrophotometer.

Deuterium lamps: These are used to create wavelengths within the 190 to 365 nm wavelength range. They are a relatively expensive consumable, so that their life is often guaranteed. Many lamps will deteriorate over time, even if they are not used.

Dissolution accessories: Controlled release tablets/capsules need to be tested to ensure that they do release the active pharmaceutical ingredient/s or nutrient/s at the required rate. Accessories include dissolution bath systems which involve dissolution vessels, paddles, tester sinkers/baskets, eight channel peristaltic pumps, eight position automatic cell changer and the appropriate flow cells and dissolution software.

Multi cell changers: These can be useful, especially when more than one sample is to be measured within a short time space, or over a given period, and the process is to be repeated. These changers may be incorporated with temperature controller devices.

PC use and control: Programmes are available to control spectrophotometers and to export spectrophotometer data to a PC/laptop.

Specular reflectance accessory: Specular reflectance is normally used for the measurement of samples which surface reflect light but show very little scatter. For example, the reflecting properties of a coated optical mirror may be measured.

Temperature controllers: Sometimes samples need to measure at specific temperatures. Thermoelectric Peltier devices offer a fast and temperature programmable means. Circulating laboratory water baths may also be coupled to spectrophotometer cell holders to achieve a specific cell temperature.

Tungsten halogen lamps: These are used to create wavelengths within the 320 to 1,100 nm wavelength range.

Xenon lamps: These are used to create wavelengths within the 190 to 1,100 nm wavelength range. They are a relatively expensive consumable, so they are often incorporated in 'press to read' technology, so they are only switched on when a measurement needs to be made. However, they do not emit good energy levels within the whole of the 190 to 1,100 nm wavelength range, and they often emit an irritating high-pitched noise.

7.RESULTS :

METHOD DEVELOPMENT AND VALIDATION BY ULTRAVIOLET SPECTROPHOTOMETRY

Determination of λ_{max} of Temozolomide

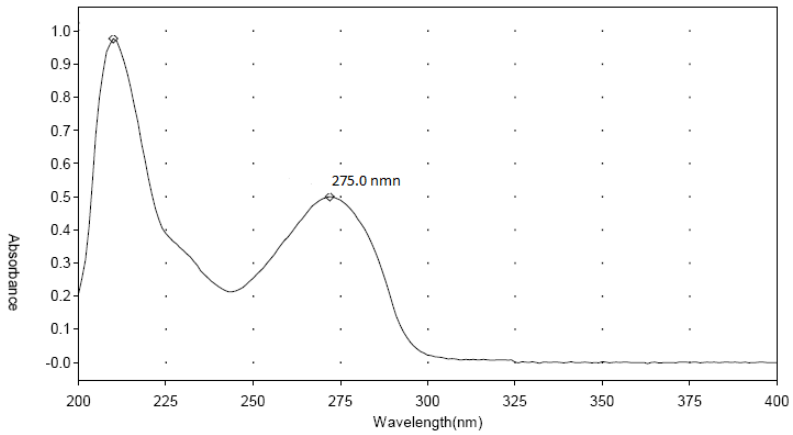


Figure 2: λ_{max} of Temozolomide – 275 nm

Method Validation

Linearity

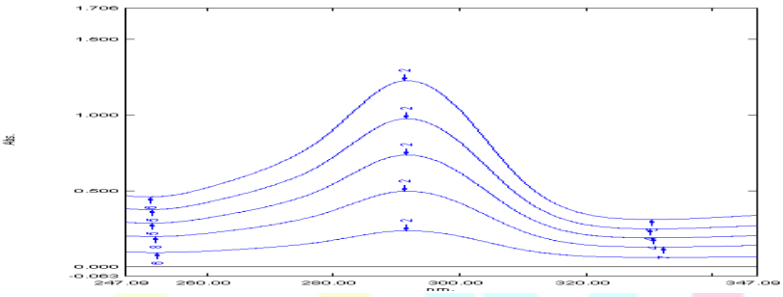


Figure 3: Overlay Spectra of Temozolomide (2-10 µg/ ml)

Table 2 : Linearity Profile by UV Spectrophotometry

Concentration (µg/ ml)	Absorbance at 275 nm
2	0.175
4	0.356
6	0.586
8	0.772
10	1.029

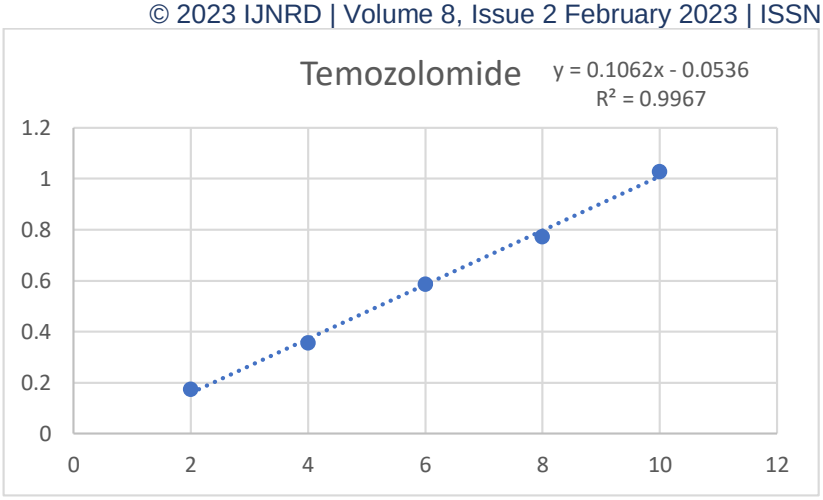


Figure 4 : Standard Calibration Curve for the Linearity Set at 275 nm by UV Spectrophotometry

Table 3: Summary of

Line equation	y= 0.1062x-0.0536
correlation coefficient (R^2)	0.9967
y- intercept (C)	0.0536
Slope (m)	0.1062

Regression Equation by UV spectrophotometer

Precision

Intra-day precision (Repeatability)

Table 4: Intra-day Precision Day- I by UV Spectrophotometry

Co(µg/ml)	Absorbance			Average	SD[a]	% RSD[b]
	Set 1	Set 2	Set 3			
2	0.175	0.177	0.174	0.175	0.0021	1.19
4	0.356	0.358	0.353	0.353	0.0021	0.59
6	0.586	0.589	0.581	0.585	0.0031	0.52

[a]= Standard Deviation, [b]= Percentage Relative Standard Deviation

Table 5: Intra-day Precision Day- II by UV Spectrophotometry

Conc (µg/ ml)	Absorbance			Average	SD	% RSD
	Set 1	Set 2	Set 3			
2	0.173	0.177	0.174	0.175	0.0021	1.19
4	0.351	0.355	0.352	0.353	0.0021	0.59
6	0.582	0.588	0.584	0.585	0.0031	0.52

Table 6: Intra-day Precision Day- III by UV Spectrophotometry

Conc (µg/ ml)	Absorbance			Average	SD	% RSD
	Set 1	Set 2	Set 3			
2	0.175	0.172	0.173	0.173	0.0015	0.175
4	0.356	0.35	0.352	0.353	0.0031	0.356
6	0.586	0.584	0.582	0.584	0.0020	0.586

6.1.2.2.2 Inter-day precision (Ruggedness)

Table 7: Inter-day Precision by UV Spectrophotometry

Conc (µg/ ml)			Absorbance				Average	SD	% RSD
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6			
2	0.175	0.177	0.174	0.175	0.172	0.173	0.174	0.0018	1.00
4	0.351	0.356	0.352	0.356	0.350	0.352	0.353	0.0026	0.73
6	0.585	0.589	0.584	0.586	0.584	0.582	0.585	0.0024	0.40

✓ The developed method was found to be precise as the % RSD of the results within and amidst 3 days was within limits (< 2.0).

- **Limit of Detection**

LOD= 3.3 * SD/Slope

= 3.3× 0.0015/ 0.1062

= 0.046 µg

- **Limit of Quantitation**

LOQ= 10 * SD/Slope

= 3.3× 0.0015/ 0.1062

= 0.141µg

✓ The obtained results were satisfactory.

Accuracy

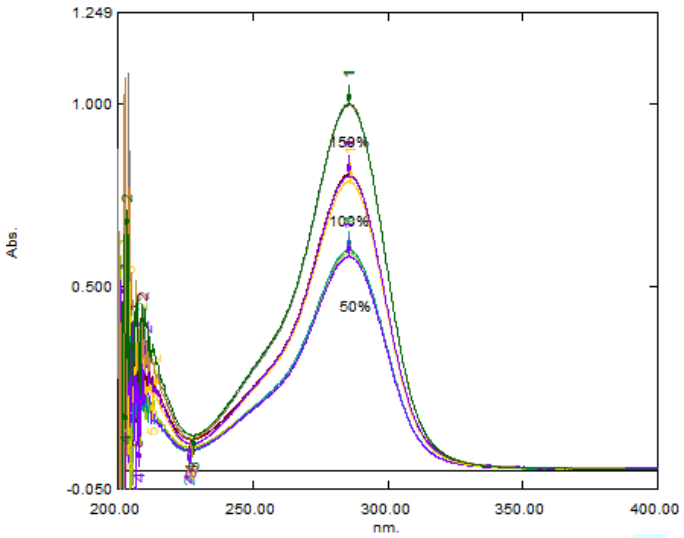


Figure 5: Overlay Accuracy Spectra of TEMOZOLOMIDE by UV Spectrophotometry

Table 8: Recovery from Formulation (Temozolomide tablets) by UV spectrophotometry

*Average of 3 experiments

Temozolomide in dosage form (µg/ml)	% Pure Temozolomide added	Pure Temozolomide Added (µg/ml)	Temozolomide Recovered% ± %RSD*
5	50%	2.5	100.02± 1.17
5	100%	5.0	100.90± 1.12
5	150%	7.5	101.00± 0.17

Acceptance Criteria: The % Recovery for each level should be between 98.0 and 102.0%

- ✓ The developed method was found to be accurate since % recovery for each level was within limits and RSD less than 2.0.

Robustness:

Table: 9 Robustness by UV Spectrophotometry

Wavelength (nm)	Absorbance						Avg	SD	% RSD
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6			
265 nm	0.169	0.171	0.171	0.170	0.172	0.169	0.170	0.0012	0.71
275 nm	0.375	0.372	0.371	0.370	0.374	0.372	0.372	0.0017	0.46
285 nm	0.567	0.561	0.565	0.562	0.566	0.564	0.564	0.0023	0.41

- ✓ *Acceptance Criteria:* The % RSD for each level should be less than 2.0%
- ✓ The developed method was found to be Robust as the % RSD of the results was within limits.

Applicability of the Developed Validated Method by Ultraviolet Spectrophotometry

Assay of formulation

Table 10: Assay of formulation (Temozolomide 75mg tablets) by UV spectrophotometry

Formulation	Absorbance	Label claim	Amount found	% Assay ± SD*
Temozolomide	0.772	75 mg	74.93	99.45%
	0.775			
	0.771			

*Average of 3 experiments

Acceptance criteria: 95- 105% w/v

- ✓ The assay results were decorous in conjunction with acceptance criteria

DISCUSSION

A simple and selective UV method is described for the determination of Temozolomide. Linearity was observed in the range 2-10 µg /ml for Temozolomide (r^2 =0.996) for the amount of drug estimated by the proposed methods was in good agreement with the label claim.

The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Temozolomide was found to be simple, precise, accurate and high resolution makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

BIBLIOGRAPHY

1. Chatwal, R. G.; Anand, K. S. High performance liquid chromatography. *Instrumental methods of chemical analysis*, 5thed.; Himalaya publishers: Mumbai, 2010; 2.570-2.629.
2. Sharma, B. K. High performance liquid chromatography. *Instrumental methods of chemical analysis*, 24th ed.; Goelpublishers: Meerut, 2005; 295 - 300.
3. Dong,W. M. HPLC instrumentation and trends. *Modern HPLC for practicing scientists*, USA, 2006; 5-10, 78-110.
4. Typical diagram of HPLC
   http://www.comsol.com/stories/waters_corp_hplc_systems/full/ Hplc diagram
5. HPLC solvent properties
   www.sanderkok.com/techniques/hplc/eluotropic_series_extended.htm
6. Swartz, M. E.; Ira Krull, S, Analytical method development. *Analytical method development and validation*, 1st ed.; Marcel Dekker, Inc: New York, 2009; 17-80.
7. Satinder, A.; Dong, M. W. Method development and validation. *Pharmaceutical analysis by HPLC*, 15th ed.; New York, 2005; 16-70.
8. Snyder, R. L.; Kirkland, J. J.; Glajch, L. J. Getting Started. *Practical Method Development*, 2nd ed.; New York, 1997; 30-100.
9. Selection of buffers
   http://www.sigmaaldrich.com/etc/medialib/docs/Aldrich/General_Information/labbasics_pg144.Par.0001.File.tmp/labbasics_pg144.pdf.
10. ICH, *Text on Validation of Analytical Procedures*, ICH – Q2A, International Conference on Harmonisation, IFPMA, Geneva, 1995, 2-3, A–1 to A–3.
11. ICH, *Validation of Analytical Procedures: Methodology*, ICH – Q2B, International Conference on Harmonisation, 1996, 1-3.
12. ICH Guidelines, Q2 (R1) - Validation of Analytical Procedures: Text and Methodology,2005, 1-6.