Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

Formulation of Baclofen Microemulsion and Validation of Analytical Method for Quantitative Estimation by UV Spectroscopy

Mohini Patidar, Nitin Deshmukh, Nilesh Mandloi, Bhoopendra Patidar

Department of Pharmaceutical Chemistry, GRY Institute of Pharmacy, Borawan, India.

(Received: 27 October 2023 Revised: 22 November Accepted: 26 December)

KEYWORDS

Baclofen, Microemulsion, Analytical method, 0.1 NaOH, Tween 80, Castor oil, Polyethylene glycol

ABSTRACT:

Introduction: Baclofen is a centrally acting antispasmodic drug used to treat muscular spasticity by acting on SNP and multiple sclerosis. Baclofen is hydrophilic in nature, can't cross the BBB, and results have 60% bioavailability.

Objectives: The objectives of the current study were to formulate a microemulsion of Baclofen in order to increase its bioavailability and to develop, optimise, and validate an analytical method for the estimation by UV-Visible spectrophotometer.

Methods: Four different batches were formulated on the basis of S/Cos ratios 3:1, 3.5:1, 4.5:1, and 1.5:1 respectively by water titration method. Tween 80, Span 80 and PEG 400 were blended together to form a homogeneous mixture, Baclofen was dissolved in oil phase. Oil phase and surfactant mixture were mixed slowly with continuous trituration for 30 minutes at 3000 rpm on a magnetic stirrer. The result of FTIR study were indicated compatibility in between Baclofen and excipients.

Results: On the basis of solubility studies, 0.1 N NaOH was selected for analytical method validation. The λ_{max} and regression equation found to be 221nm and Y=0.0603x+0.0207, R^2= 0.999. The results of all analytical validation parameters were < 2% RSD value. Out of all batches M1 shows more transparent, no phase separation, pH of 6.7, particle size 100nm, low viscosity and 96.73% drug content value. It exhibited good stability over the course of the six-month stability study, with no significant changes in its physicochemical characteristics.

Conclusions: On the basis of the finding, it was concluded that M1 batch is a feasible, effective and it boosts the bioavailability of Baclofen.

1. Introduction

Hoar and Schulman first proposed a microemulsion concept in 1943 as a kind of NDDS technological advancement [1]. A microemulsion is uniform, transparent and isotropic dispersions of O/W or W/O type of formulation which is stabilized by combinations of oil, water, surfactant and co-surfactant [2]. The aqueous phase may also contain salt(s) and/or other chemicals, whereas the "oil" is a complicated mixture of different hydrocarbons and olefins. Contrary to typical emulsions, microemulsions are formed simply by mixing the ingredients and do not require the high shear conditions that are frequently used to generate normal emulsions. Oil distributed in water is known as a direct microemulsion, and water dispersed in oil is known as a reverse microemulsion. In microemulsion surfactant molecules may form a monolayer at the interface between the oil and water when two immiscible phases (water and "oil") are present in ternary systems. Small droplets or particles in the dispersed phase typically present in the range of 10 to 200 nm. [3, 4]. (Fig No.1)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

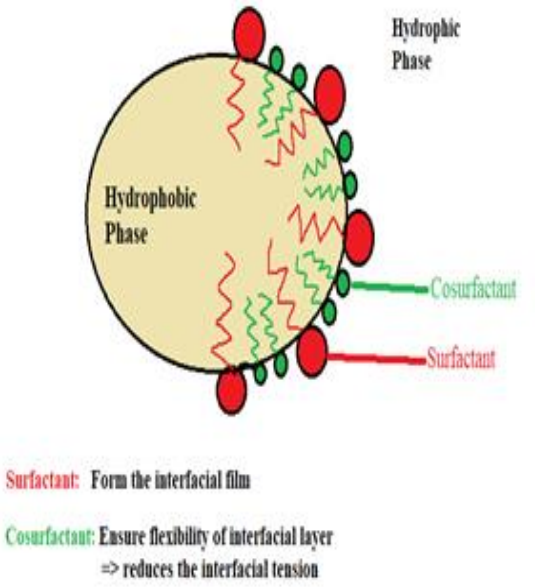

Figure 1: Structure of Microemulsion

Microemulsions are suitable for the administration of drugs via percutaneous, oral, topical, transdermal, ocular, and parenteral routes as they provide for a prolonged or controlled release [5].

Baclofen (γ-amino-β-[p-chlorophenyl]-butyric acid) is a centrally acting skeletal muscle inhibiter act on γ-aminobutyric acid (GABA) neurotransmitter, available in tablet, solution, packet, and suspension formulations. It is used to treat muscle stiffness, spasticity, particularly in people with multiple sclerosis or neurological conditions [6]. Drug has 2-6 hours half life and 60% oral bioavailability because it's hydrophilic properties; due to this it does not readily cross the blood-brain barrier. 70-80% of Baclofen is eliminated in an unchanged form by renal excretion. Baclofen is prescribed to take TDS because the cerebrospinal fluid has drug concentrations of the drug that are roughly 8.5 times lower than those in the plasma [7].

2. Objectives

In this study, a Baclofen o/w microemulsion was formulated using polymer, oil, and surfactant to increase bioavailability and improve drug absorption across biological membranes. Low viscosity microemulsions have greater absorption into the body, less surfactant content decreases negative effects, and improve solubility of drug which increases the bioavailability of Baclofen.

A number of analytical methods are available to assess the purity of the Baclofen through UV spectroscopy by using different solvents [8]. Another goal is to develop, optimize, and validate novel analytical method which is quick, simple, precise, sensitive and cost effective for estimating Baclofen using a UV-visible spectrophotometer.

3. Material and Methods

Instrumentation

UV-1800 shimadzu with UV probe software system were utilized for qualitative determination of Baclofen, Digital PH meter, Laboratory Centrifuge, Brookfield Viscometer, Stage Microscope, Magnetic Stirrer, Pycnometer.

Reagents and Chemicals

Baclofen was purchased from Yarrow Pharma Mumbai, Tween 80 was purchased from Sd Fine chemicals Ltd, Ahmadabad, Span 80 and PEG 400 were purchased from Loba chemicals Ltd Mumbai, Castor oil was purchased from Gliter Pharmaceutical Rangwasa Rao, Each of the ingredients and chemicals are an analytical grade. They were procured from the GRY Institute of Pharmacy's laboratory in Borawan, India.

Pre-formulation studies

Drug-excipients compatibility study

FTIR is an effective method for examining the interaction between drugs and excipients. For this study surfactant, co-surfactant, and oil were added to the pure medication individually then KBr pellets are prepared and the resulting mixture was scanned in the 400–4000 cm-1 range. Drug-excipients FTIR graphs were compared with pure Baclofen graph [9].

Solubility analysis

A solubility analysis was carried out in water, ethanol, methanol, 0.1N HCl, and 0.1N NaOH solution to create a new analytical method. Baclofen powder was added in excess to the solvents and the mixture was then vortexed. The undissolved drug was subsequently removed from the equilibrated samples using sonication for 30 minutes. Filter the solution and view transversely, against a black background. On the basis of solubility analysis appropriate oil, surfactant, and co-surfactant were chose.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

Construction of phase diagram

The pseudoternary phase diagram construction was one of the important studies for formulation microemulsion.

On the basis of solubility study Tween 80, Span 80 was used as a surfactant and cosurfactant (Smix). Smix were prepared by mixing weight rations of 1:1, 1:2, and 2:1. There were 2 oils castor oil and sesame oil were selected as an oil phase. Mix castor oil and Smix at weight ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1.Titrate the mixtures with distilled water until the formation of transparent, free-flowing mixtures which indicate equilibrium point. The volume of the water used was recorded. The composition of each component at the equilibrium point was determined and calculated into a weight percent. The pseudoternary phase diagrams were constructed using ternary plot software. Same procedure was repeated with sesame oil. Compare the result of both the oil: Smix rations [10].

Description of analytical method

Selection of wavelength

100mg pure Baclofen was dissolved in 100 ml of 0.1N NaOH solution to create a primary stock solution with a concentration of 1000µg/ml. Different concentrations of the drug dilution (2, 4, 6, 8, 10µg/ml) were prepared with the same solvent and then scanned with a UV-Visible spectrophotometer between the wavelengths of 200-400 nm with a 0.1N NaOH solution serving as a blank.

Validation of analytical method *[11-13]*

a. Accuracy

From the stock solutions 4, 6 and 8µg/ml concentration dilution were made in 0.1 N NaOH and samples were scan at 221nm to measure the accuracy. From the absorbance value purity % was determined and calculates SD and RSD value.

b. Precision

The precision was determined in the terms of repeatability, intermediate and reproducibility. 4, 6 and 8µg/ml concentration solutions were analysed to determine all parameter and the relative standard deviation was calculated.

c. *Linearity*

A series of dilutions of Baclofen at 1, 2, 3, 4, 5, 6, 7, 8, and 10µg/ml were made and then scanned under a UV light. A calibration curve was created using the resulting absorbance data. Further calculations were made by Y-intercept equation's correlation coefficient.

d. Determination of Limit of Quantitation (LOQ) and Limit of Detection (LOD)

To assess the sensitivity of the suggested method, LOD and LOQ were calculated as per the ICH recommendations.

e. Range

By comparing the results interval of lower and upper levels of LOD, it has been possible to define the range of analytical methods with a reasonable degree of precision and accuracy.

f. Robustness

The robustness of this method was determined by analyzing of 3 and 6 µg/ml concentration solutions within the day and calculating SD and RSD.

g. Ruggedness

The robustness of this procedure was assessed by two analysts analysing solutions with concentrations of 5 and 10µg/ml on the same equipment. After that, SD and RSD were calculated.

Formulation of Microemulsion

For the formulation of o/w microemulsion castor oil and sesame oil were chosen as the oil phases, tween 80, span 80 as a surfactants and co-surfactants; propylene glycol as a polymer used. In the current study, four composition formulations of surfactant-cosurfactant ratios (S/Cos ratios) batches, including 3:1, 3.5:1, 4.5:1, and 1.5:1, were evaluated. In the oil phase, Baclofen was dissolved, and surfactant was subsequently added in separately co-surfactant and PEG were blended together to form a homogeneous mixture (Smix). Then oil phase and Smix phase were mixed slowly with continuous trituration. Drops of water were added while swirling continuously for 30 minutes at 3000 rpm on a magnetic stirrer. The microemulsions that were formulated have equilibrium between liquid-water phases, milky white, turbid, translucent, and clear biphasic solution [2,4]. [Table 1]

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

Table 1: Composition Formula of Baclofen Microemulsion

Ingredients	Formulation code (100 ml)				Purpose
	M1	M2	M3	M4	
Baclofen	100 mg	100 mg	100 mg	100 mg	Drug
Tween 80	22 ml	18 ml	18 ml	22 ml	Surfactant
Span 80	22 ml	28 ml	28 ml	22 ml	Co-surfactant
PEG 400	8 ml	8 ml	8 ml	8 ml	Emulsifier
Sesame Oil	-	6 ml	7 ml	12 ml	Oil phase
Castor Oil	12 ml	6 ml	7 ml	-	Oil phase
Rose water	4 ml	4 ml	4 ml	4 ml	Fragrance
Water	32 ml	30 ml	30 ml	32 ml	Aqueous phase

Evaluation Parameters of Baclofen Microemulsion

Optical Transparency

By visual inspection physical properties like colour, phase separation, creaming and clarity of formulation were examined [14].

Viscosity measurement

Using a Brookfield Viscometer, the optimised formulation's viscosity was assessed without dilution (DV-E Brookfield Viscometer Model-LVDVE). 500ml sample formulation was keeping in beaker, at 35^0 C ±2 room temperature and Spindle no. 6, 7 at 60 rpm was used to measure the viscosity. For reproducible results, these processes were repeated three times [4].

Particle size determination

The stage microscopic method is used to determine particle size. In this technique, a created microemulsion droplet was placed on a piece of glass using a stage microscope. A 100x eyepiece lens was used to observe the droplet's size and then particle size was calculated [3, 15].

Specific gravity

The density of the microemulsion and the water was obtained by using a density bottle, and the specific gravity of the microemulsion was then calculated using the formula [16].

pH determination

The pH of the microemulsion was measured using a digital pH meter at 25ºC. For greater accuracy, every reading was obtained in triplicate, and the estimate of the triplicates was obtained [17].

Drug content analysis

In 100 ml of 0.1N NaOH, 5ml of the Baclofen microemulsion were dissolved, shake the solution till the microemulsion is completely mixed, then kept in centrifugation tube at 3000rpm for 30 minutes. The surplus was separated and filtered. Dilute 1ml of filtrate solution to 10 ml of solvent, then filtered one's more. Analysed the test solution's absorbance at 221 nm using placebo microemulsion as a control, and the drug content was estimated [4, 17].

Stability testing

According to ICH guidelines, the accelerated ambient stability investigation was carried out at humidity (75 ± 5%RH), temperature 40 ± 2 °C, 25°C and 10°C, respectively for 6 month duration. The samples of formulation were kept in a hermetically screw-capped bottle with a volume of around 100 ml. The physical and chemical properties of samples were analysed in every three month intervals [3, 4, 18].

4. Results

Interpretation of IR Spectra of Baclofen and other excipients

Characteristic absorption bands in the IR spectra of Baclofen can be identified. They are connected to stretching vibrations of the N-H (3447.89cm1), C-Cl (664.48cm1), and COOH (1729.65cm1) vibrations. Similar specific bands have been found in the IR spectra of Baclofen-excipients mixtures. Therefore, it served as evidence that Baclofen and excipients were compatible. (Fig No.2,3)

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

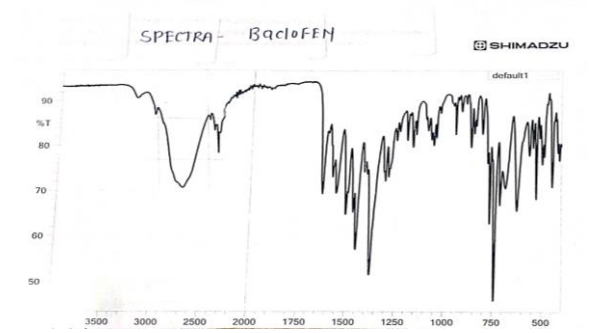

Figure 2: Baclofen IR Spectrum

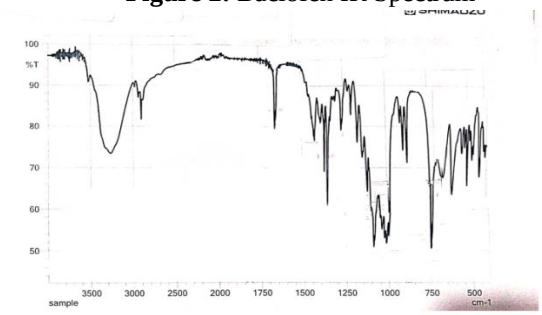

Figure 3: Baclofen microemulsion IR Spectrum

Solubility

Baclofen shows better solubility with 0.1 N NaOH so it is used as solvent for analytical method development. [Table 2]

Table 2: Solubility Study

Solvent	Solubility
Water	Slightly soluble
Ethanol	Partial soluble
Methanol	Partial soluble
0.1 N NaOH	Soluble
0.1 N HCl	Soluble

Construction of pseudoternary phase diagram

The pseudoternary phase diagram was created to determine the concentration range of components. In the phase diagrams, translucent o/w microemulsion region was shown as a dot region. Different composition formulas were developed in accordance with the results of the phase diagram. While oil level was high in M3 formulation, water level is higher in M1 and M4. Smix, cosurfactant, and individual surfactants were investigated for their effects. Transparent o/w microemulsions should have water in the 60–70% range, Smix in the range of 1:1, and oil in the 10-12% range.
(Fig No.4)

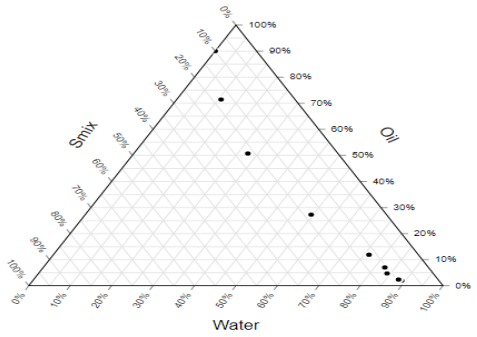

Figure 4: Phase Diagram

Analytical Method Results

Different dilutions ranging from 2 g/ml to 10 g/ml were prepared for the purpose of determining the analytical wavelength and scanned at wavelengths between 200 and 400 nm in a UV-Visible spectrophotometer. Maximum absorbance was detected in the dilutions at 221 nm. Consequently, 221 nm was chosen as the analytical wavelength.

Validation of Analytical Method

The correlation coefficient equation was determined to be y = 0.0603X + 0.0207 and the regression value was found to be R^2= 0.999 based on the results of linearity values. (Fig No.3) Accuracy, precision, robustness and ruggedness were calculated and result value of the relative standard deviation was not exceeding by 2%. Results of LOD, LOQ, and range were found to be 0.825µg/ml, 2.5µg/ml, 1µg/ml-10µg/ml respectively. [Table 3] (Fig No.5,6)

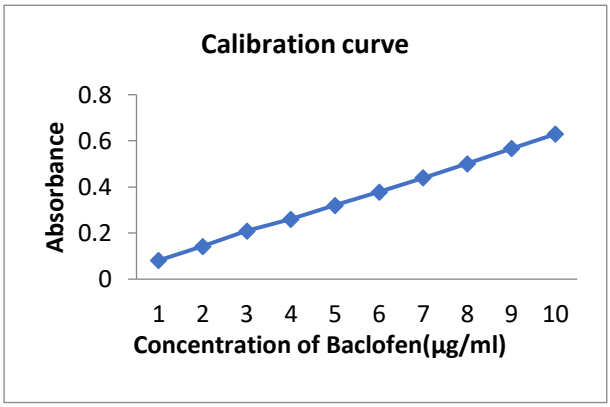

Figure 5: Calibration curve results

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

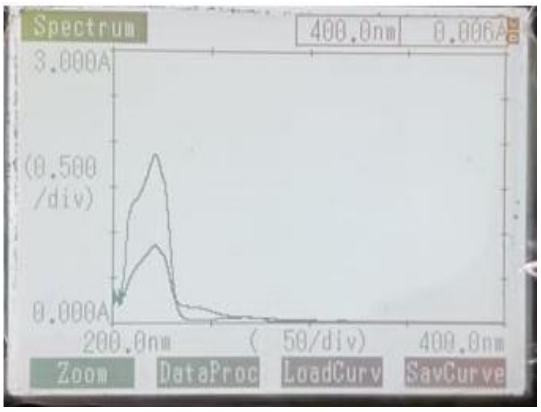

Figure 6: Overlain UV Spectrum of Baclofen in 0.1 N NaOH solvent

Table 3: Summary of Validation Parameters

S. N.	Parameter	Results
1	λmax	221nm
2	Regression Equation (Y=mX+C)	Y=0.0603X+0.0207
3	Slope	0.0603
4	Intercept	0.0207
5	Correlation Coefficient (R^2)	0.9995
6	Accuracy (RSD)	Less than 2%
7	Interday precision (RSD)	Less than 2%
8	Intraday precision (RSD)	Less than 2%
9	Linearity range	1µg/ml-10µg/ml
10	LOD	0.825µg/ml
11	LOQ	2.5µg/ml
12	Ruggedness1	Less than 2%
13	Ruggedness	Less than 2%
14	Robustness	Less than 2%
15	%Purity of formulation	99.3%

Composition formula optimization

Span 80 has a low HLB value with lipophilic properties, which can be used to form oil-in-water microemulsion when used in combination with Tween 80 having hydrophilic properties and high HLB value. The emulsifier's tween 80 and span 60 are frequently used in combination. In comparison to using only one surfactant, the combination of these two surfactants can increase the solubility of drug in water and physical stability of the microemulsion. PEG-400 was used as co-solvents and polymer for improving the aqueous solubility of weakly water-soluble Baclofen which will help in improving drug bioavailability. Four different composition formulas were optimized for fixing the S/Cos ratios. [Table 4] Out of the four formulae, M1 appears as a transparent, isotropic liquid mixture of surfactant, water, and oil that is thermodynamically stable. The results of their evaluation are listed below. (Fig No.7)

Figure 7: Baclofen Microemulsion Formulations

Table 4: Result of Evaluation Parameters

Parameters	Formulations			
	M_1	**M_2**	**M_3**	**M_4**
Phase separation	No	No	Yes	Yes
Clarity	Transparent	Milky	Milky	Milky
Colour	Yellowish	Brown	White	Whitish Brown
Viscosity (dynes/cm^2)	82	87	95	110
Particle size(nm)	100	110	118	125
Specific gravity	1	0.89	0.8	0.6
pH	6.7	6.2	6.4	6.5
Drug content	96.73%.	90.32%	89.19%	92.45%

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

Result of formulation evaluation parameters

Optical Transparency

Microemulsions are regularly inspected to check the formulation's colour change, phase separation in oil and water phase, upward and down ward creaming. Through the examination of a physical parameter it was concluded that there is no colour change and creaming of formulations found. Both the phase's oil and water were immiscible with each other. Microemulsion of Baclofen M1 was transparent and cleared solution.

Viscosity measurement

Using a Brookfield Viscometer, all the formulation's viscosity was assessed at 35^0 C±2 room temperature and 60 rpm. Viscosity of formulation M1 & M4 were found to be 82 and 110 dynes/cm^2.

Particle size determination

The radius of the globules is directly proportional to the rate of phase separation. The formulation's particle size has been found to be within an acceptable range, 100-125 nm. (Fig No.8)

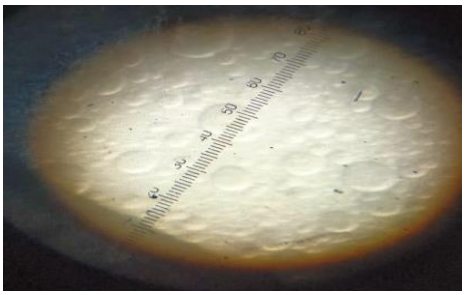

Figure 8: Baclofen Microemulsion Particle size

Specific gravity

The density of the microemulsion was calculated as compared to water. The specific gravity of the microemulsion was found to be 0.89-1.00, equivalent to water.

pH determination

The pH of the microemulsion was measured using a digital pH meter. The pH of the formulation M1 found to be 6.7, which in slightly acids in nature.

Drug Content Analysis

For each batch of formulation, drug content studies were completed in triplicate. The calculated drug content was found to be in range of 89.19% to 96.73%. According to the data, formulation M3 has the least drug, whereas formulation M1 contains the highest amount shown in.

Stability Testing

The stability study was carried out to refine the ideal microemulsion formulation for harsh environments. After 3 month periods sample was collected and evaluated. Drug content, pH and viscosity of best formulation M1 was 95.98%, 6.6 and 81.4 respectively. There is no phase separation, cracking, and coalescence was examined in the samples.

5. Conclusion

A microemulsion of Baclofen was successfully formulated. Microemulsions have improved the bioavailability, effectiveness, and rate of absorption of Baclofen in accordance with the objectives of the current investigation. The optimized formula consists of Tween 80, Span 80, and PEG 400 in (3:1) ration. A FTIR investigation found that the excipients and the medication were compatible. Utilising 0.1 N NaOH solutions the analytical method was validated and it was accurate, precise, and economical. M1 batch had all the desired characteristics and a drug content of 96.73% across all batches. Based on the findings, it was determined that the microemulsion formulation of Baclofen is a feasible and effective for oral deliver that will boost bioavailability.

Acknowledgements

Without the assistance of Dr. Sujit Pillai, Mr. Sumit Patel, Ms. Aakansha Mahajan and my family, this research would not have been feasible. I'd like to take this initiative to thank GRY Institute of Pharmacy for providing the required equipment and materials to enable me to conduct the research project.

Abbreviation

NDDS: Novel Drug Delivery system, **FTIR:** Fourier Transform Infrared Analysis, **UV Spectroscopy:** Ultra Violet Spectroscopy, **SD:** Standard Deviation, **RSD:** Relative Standard Deviation, **σ:** Slope, **PEG:** Polyethylene Glycol.

Refrences

1. Gibaud, S., Attivi, D., 2012. Microemulsions for Oral administration and their Therapeutic applications. Expert Opinion on Drug Delivery.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(1), 2773-2780 | ISSN:2251-6727

9(8),937–951.
DOI:10.1517/17425247.2012.694865

2. Rao S, K. D, Chowdary K., 2009. Microemulsions: a Novel Drug Carrier System. International Journal of Drug Delivery Technology. 1(2),39-41.
3. S. Madhav and D. Gupta, 2011. A Review on Microemulsion Based System. IJPSR. 2(8),1888-1899.
4. Saini J, Nautiyal U, Kumar S, Singh D, Anwar F., 2014. Microemulsions: A Potential Novel Drug Delivery System. International Journal of Pharmaceutical and Medicinal Research. 2(1), 15-20.
5. Katiyarsarvesh B, Katiyarsarvesh S., 2013. Microemulsions: A Novel Drug Carrier System. International Journal of Pharmceutical science. 20(2),138.
6. Baclofen, Drug Bank. Available from: https://go.drugbank.com/drugs/DB0018.
7. Rojek B, Wesolowski M, Suchacz B., 2013. Detection of Compatibility between Baclofen and Excipients with Aid of Inhrared Spectroscopy and Chemometry. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 116, 532–538.
8. Gabhane KB, Jaiswal A, Tapae KK., 2014. Simple and Validated Ultraviolet Spectrophotometric Method for the Estimation of Baclofen in Bulk Form. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 5(6), 104.
9. Lotfi B, Ghorbel A, HassineT., 2007. Baclofen Pharma Profile. Article in Compendium Yardley, PA. 629-623.
10. Padhiar J S, Shirolkar S V, Deshkar S S., 2018. Formulation And Evaluation of Microemulsion Based Topical Gel of Carbamazepine. International Journal of Drug Delivery Technology. 8(2), 44-52. DOI: 10.25258/ijddt.v8i2.13867.
11. Adhao V, Thenge R., 2017. Development and Validation of Stability Indicating High Performance Liquid Chromatography Method for Determination of Baclofen. American Journal of Pharmtech Research. 7(2), 544.
12. Potdar M., 2017. Pharmaceutical Quality Assurance. Nirali Prakashan. (6)8, 29-8.31.
13. ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology, Geneva: IFPMA.1996: 1-5.
14. Katiyar B, Katiyar S., 2013. Microemulsions: A Novel Drug Carrier System. International journal of Pharmceutical science. 20(2), 138.
15. Gui Sh Y, Wu L, Peng D Y, Liu Q Y, Yin B P, Shen J Zh., 2008. Preparation and Evaluation of A Microemulsion for Oral Delivery of Berberine. Pharmazie. 63, 516–519.
16. Yadav V, Jadhav P, Kanase K, Bodhe A., 2018. Preparation and Evaluation of Microemulsion Containing Antihypertensive Drug. International Journal of Applied Pharmaceutics. 10(5), 138-146.
17. Panghal A, Sachdeva M, Agarwal V., 2022. Formulation & Development of Baclofen Microemulsion Incorporated into Transdermal Patch. Journal of Drug Delivery & Therapeutics. 12(5), 55-63.
18. Nath R, Bhowmik R, Chakraborty R, Datta S and, Chakraborty A., 2021. Preparation and Evaluation of a Novel Oral Microemulsion Drug Delivery System for Enhancing the Bioavailability of Diltiazem. International Journal of Pharmaceutical Sciences Review and Research. 70(2), 164-170.
19. Badawi A, Nour S, Sakran W, Sameh S., 2009. Preparation and Evaluation of Microemulsion Systems Containing Salicylic Acid. AAPS PharmSciTech. 10(4), 1081-1084. DOI: 10.1208/s12249-009-9301-7.