Applicant Name: Lloyd Institute of Management and Technology **No of Sheet:06**

Application Number: **Sheet No:01/06**

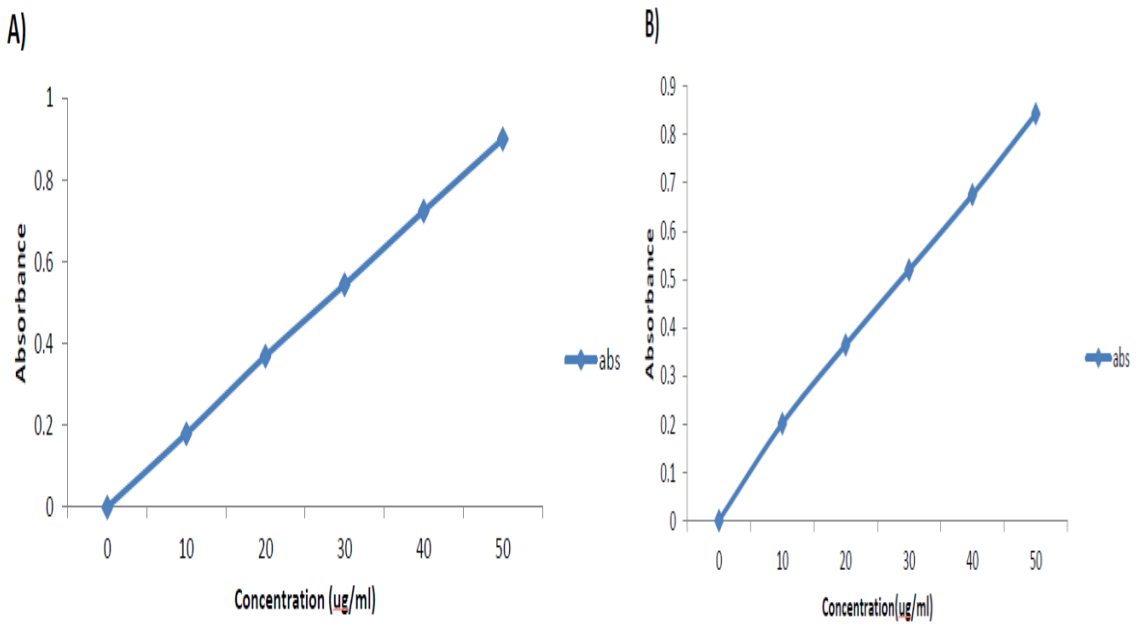

Fig 1. Calibration curve of Quetiapine: A) In Ethanol B) Phosphate buffer pH(6.4).

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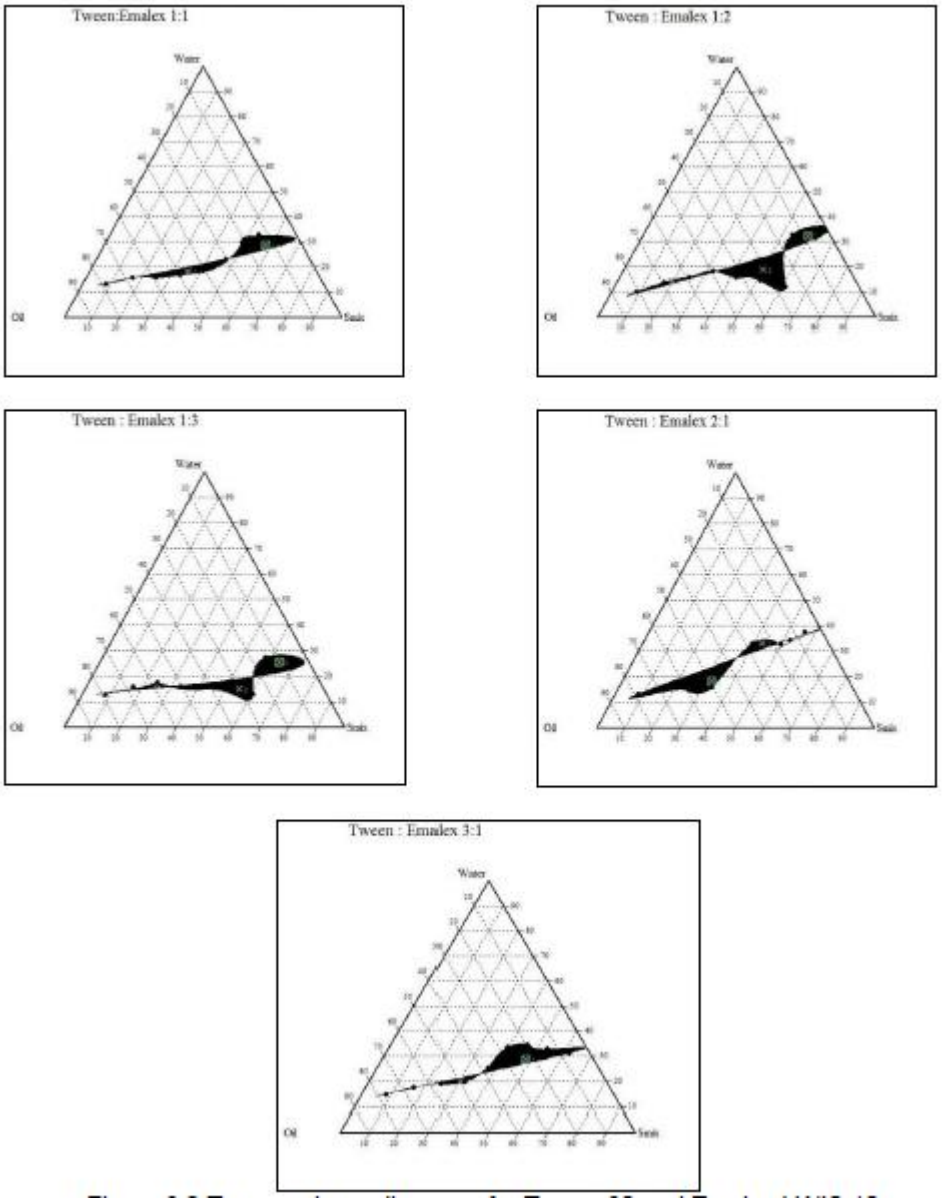

Figure 2 : Ternary phase diagrams for Tween 80 and Emalex LWIS 10.

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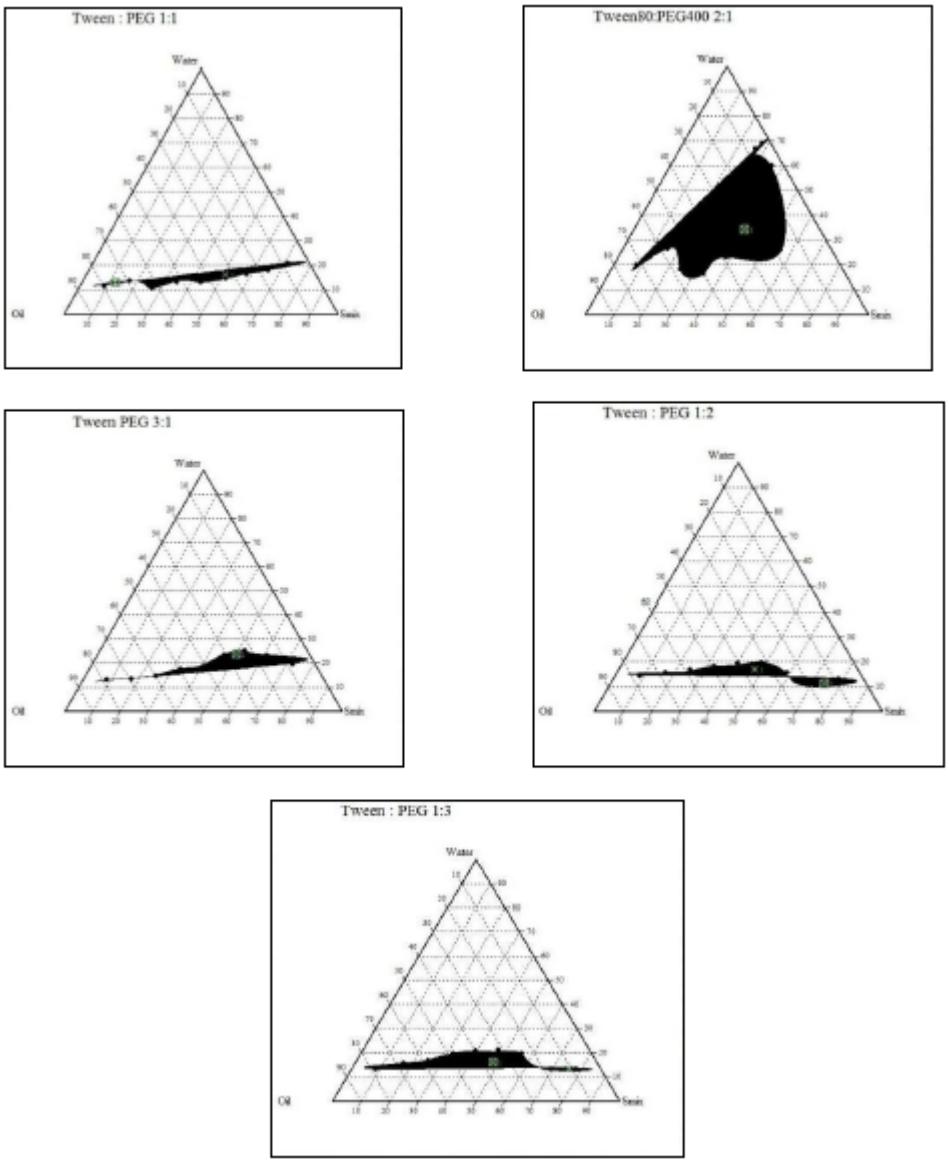

Figure 3, depicts Ternary phase diagrams for Tween 80 and PEG 400.

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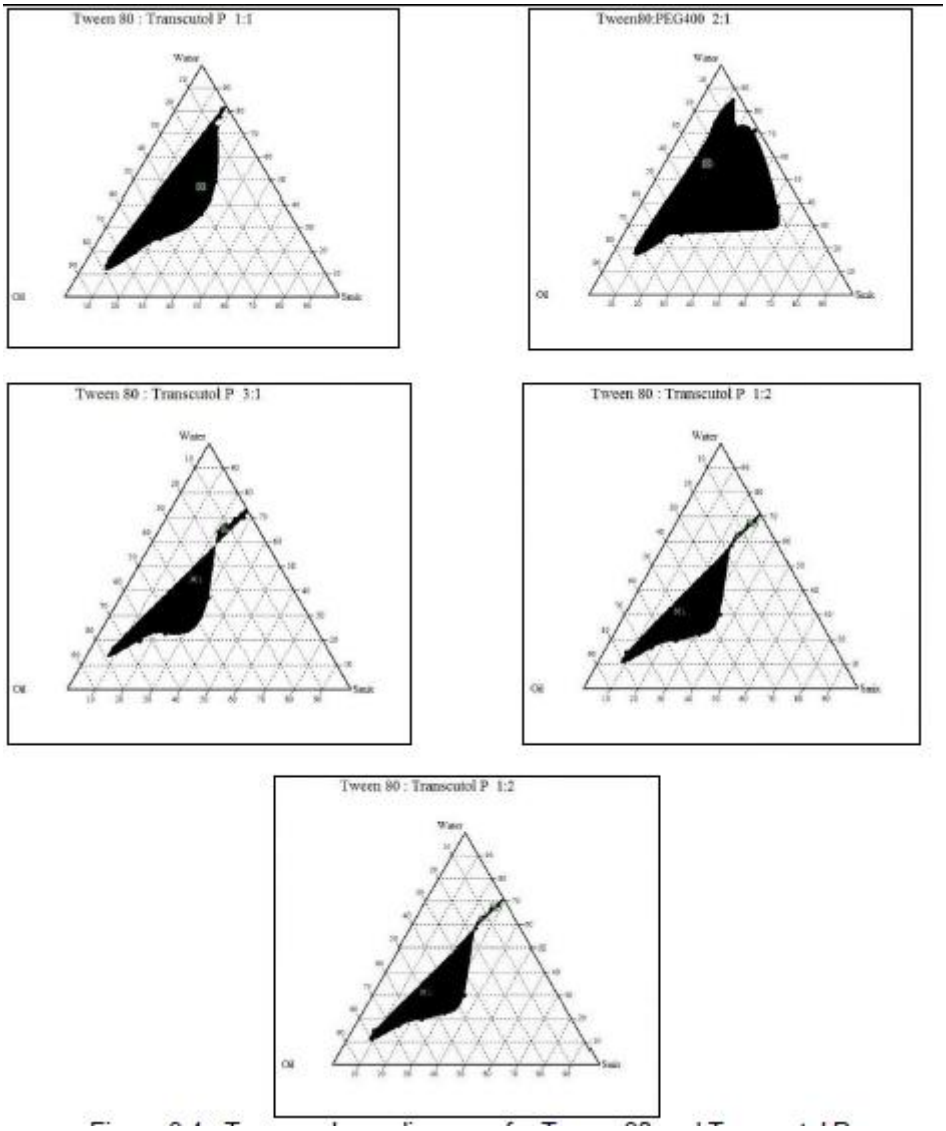

Figure 4 : Ternary phase diagrams for Tween 80 and Trnascutol P.

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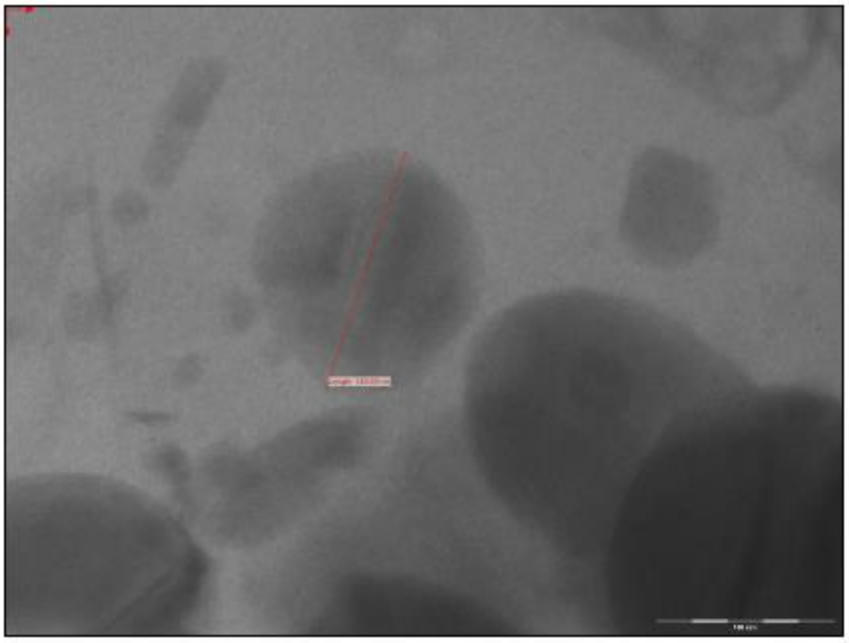

Figure 5, TEM image of nanoemulsion (Batch QP11)

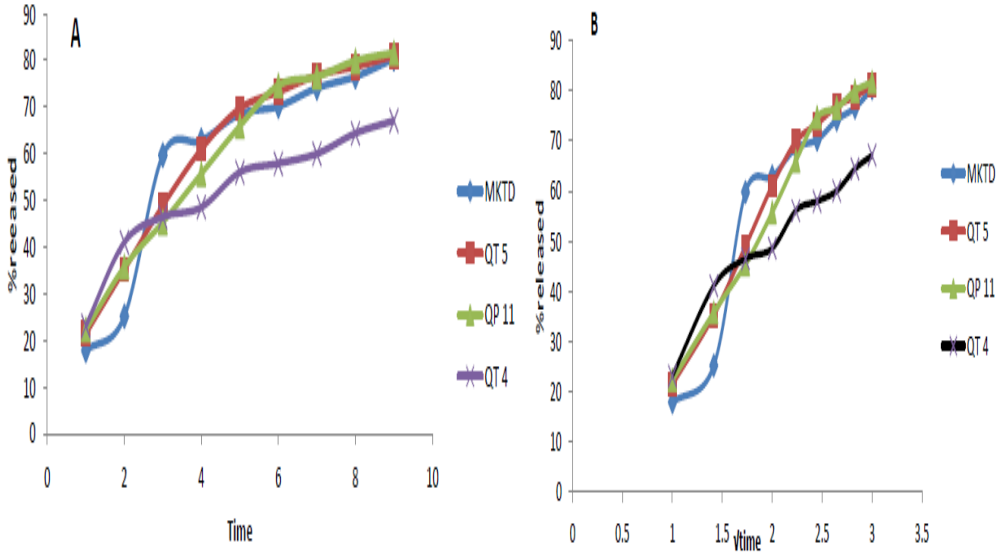

Figure 6, A) zero order: % release v/s time. B) High model: % release v/s √time.

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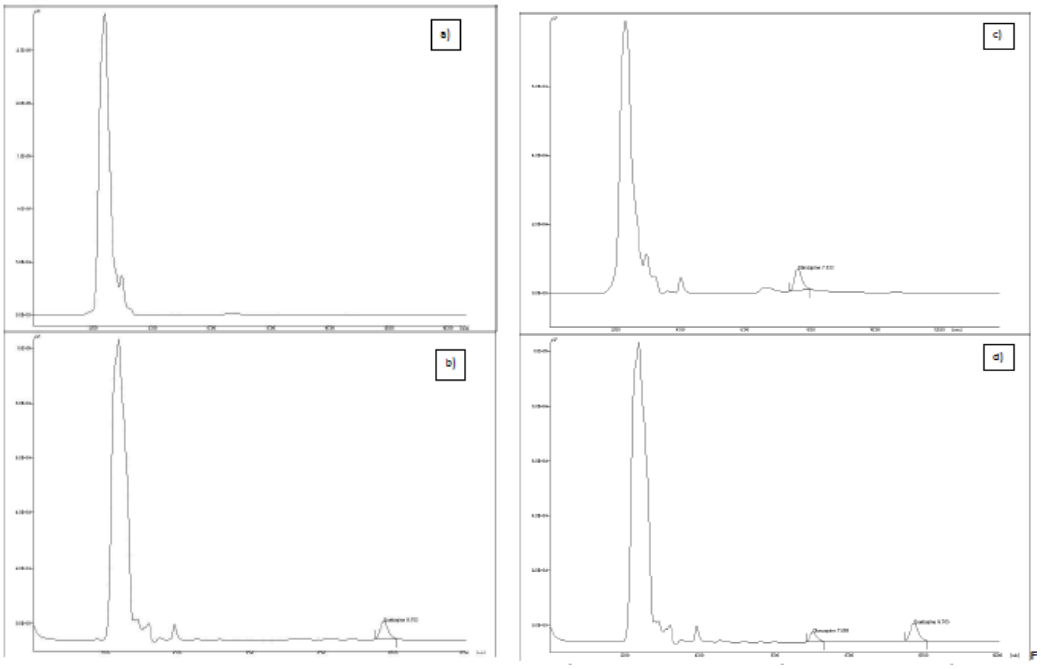
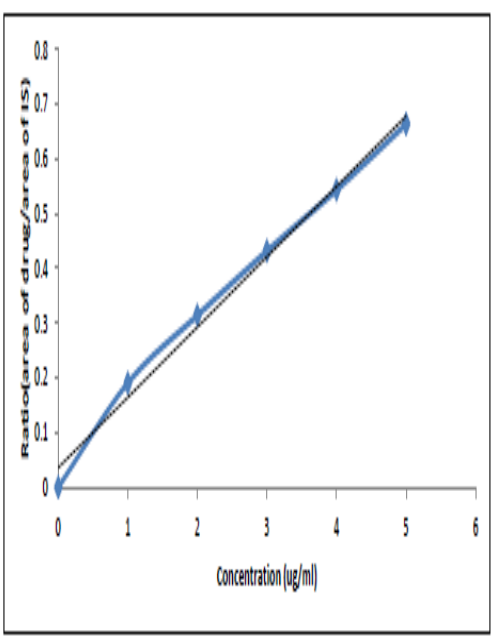


Figure 7: Chromatograms of a) pure plasma, b) quetiapine, c)olanzapine d)quetipine : olanzapine.



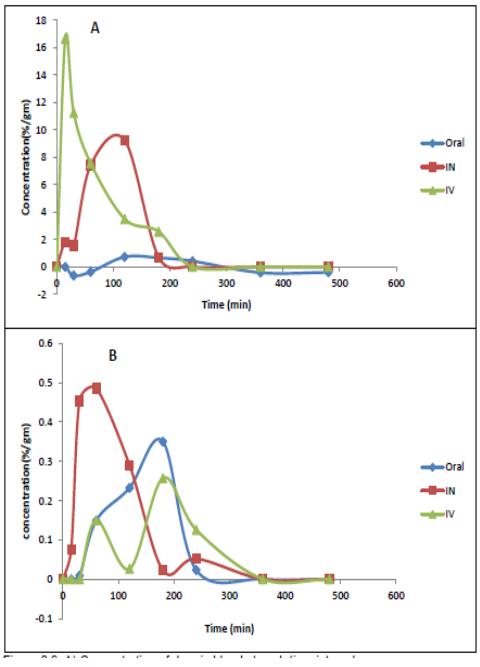


Figure 8, calibration curve of quetiapine in plasma. Figure 9, A) Concentration of drug in blood at each time interval. B) Concentration of drug in brain at each time interval

Signature

FORM-2

THE PATENTS ACT, 1970

(39 of 1970)

&

THE PATENTS RULES, 2003

COMPLETE

SPECIFICATION

(See section 10 and rule 13)

TITLE OF INVENTION

An Improved Quetiapine Nanoemulsion Formulation and Its Therapeutic Application Thereof

APPLICANT NAME AND ADDRESS

APPLICANT NAME AND ADDRESS

Lloyd Institute of Management and Technology,

Plot No.-11, Knowledge Park-II, Greater Noida, Uttar Pradesh, India-201306

THE FOLLOWING SPECIFICATION PARTICULARLY DESCRIBES THE INVENTION AND THE MANNER IN WHICH IT IS TO BE PERFORMED

FIELD OF INVENTION- The present invention relates to preparation of an improved quetiapine nanoemulsion formulation and its therapeutic application for intranasal drug delivery**.**

BACK GROUND OF INVENTION-

US11013715B2 disclose herein are compositions, having nanosized droplets, wherein the nanosized droplets may contain a cannabinoid oil, a dietically acceptable carrier oil, a surfactant, and water. Also disclosed herein are methods of making and using the same.

US20070148251A1 relates to nanoparticles and medical devices (such as, for example, a stent) containing the nanoparticles. The nanoparticles can include a bioactive agent and can further include a matrix material. The nanoparticles can be loaded onto and released from the medical device (e.g., stent) via a porous matrix, a channeled surface, a depot structure, or a stent with nanoporous or micro-depot surface. Alternatively, the nanoparticles can be included in a coating on a medical device (e.g., a drug-delivery stent coating) and released therefrom upon implantation of the medical device. In some configurations, the nanoparticles may have a shell enclosing a volume, or the nanoparticles may include a porous material which can be loaded with a bioactive agent (e.g., a small molecule drug, protein, or peptide).

US 8,101,597 B2 discloses novel and stable salt forms of quetiapine, processes for preparation, pharmaceutical compositions, and method of treating thereof. More particularly, the present invention provides novel acid addition salts of quetiapine wherein the acid counterion is provided by an acid selected from the group consisting of benzene Sulfonic acid, dibenzoyl-L-(+)-tartaric acid and di-p-toluoyl-L-(+)-tartaric acid. The present invention also provides novel polymorphic forms of quetiapine salts selected from the group consisting of quetiapine hydrobromide, quetiapine Sulfate, quetiapinenitrate and quetiapine citrate.

F.A. Araujo et al. (2011) Developed and carried out characterization of parenteral nanoemulsions containing thalidomide. Formulations prepared with castor oil (10%, w/w) lecithin (3.0%, w/w) and increasing amounts of THD (from 0.01 to 0.05%) presented droplet size, polydispersity index and zeta potential feasible to i.v. administration. Nevertheless, drug crystallization was observed for the higher content formulation and it was demonstrated that THD always precipitated in the α-polymorphic form. A significant decrease of THD content was observed for the 0.01% THD formulation at 60 days of storage and the addition of

polysorbate 80 improved the stability of this formulation only at a 0.5% (w/w) level. Higher polysorbate 80 concentrations led to a significant decrease on drug loading, possibly by facilitating drug interaction with the aqueous phase.

V. Devarajan et al. (2011) The delivery of poorly water-soluble drugs has been the subject of much research, as approximately 40% of new chemical entities are hydrophobic in nature. One area in which published literature is lacking is the field of Nano emulsions. Nanoemulsion system provides reservoir vehicles for transdermal systems and controlled drug delivery systems or hydrolytically unstable drugs. The design and development of new drug delivery systems with the intention of enhancing the efficacy of existing drugs is an ongoing process in pharmaceutical research. It is necessary for a pharmaceutical solution to contain a therapeutic dose of the drug in a volume convenient for administration.

Tushar K. Vyas et al. (2005) formulated microemulsions containing zolmitriptan (ZT) for rapid drug delivery to the brain to treat acute attacks of migraine and to characterize microemulsions and evaluate bio-distribution in rats. Zolmitriptan microemulsions (ZME) were prepared using the titration method and were characterized for globule size distribution and zeta potential. ZT was radiolabeled using 99mTc (technetium) and radio labeled-drug formulations of ZT were used to carry out bio-distribution of drug in the brain of Swiss albino rats after intranasal and intravenous administration. The pharmacokinetic parameters, drug targeting efficiency (%DTE) and direct nose-to-brain drug transport (%DTP) were calculated. Brain imaging in rats were also performed to ascertain the uptake of drug into the brain. Comparing intranasal administration of ZMME with intravenous administration of ZME, the %DTE and %DTP were found higher indicating effective drug transport following intranasal administration and highest brain-targeting following ZMME administration. Rat brain scintigrams showed substantial uptake of drug into the brain after intranasal administration of ZMME.

PROBLEM STATEMENT

There is lots of literature describing the preparation of Quetiapine, as an oral Tablet and the oral bioavailability of quetiapine is only 9%, which is having solubility problems that affects its absorption after oral administration, as it undergoes extensive first pass metabolism.

Hence the formulation should be designed for targeting of the quetiapine to the brain so as to avoid its metabolism by hepatic first pass effect which in turn can improve the bioavailability of quetiapine.

Therefore the present invention discloses the preparation of quetiapine nanoemulsions by virtue of their lipophilic nature and low globule size are widely explored as a delivery system to enhance uptake across nasal mucosa.

SUMMARY OF THE INVENTION

The present invention has been developed in response to the present state of the art, and in particular, in response to the problems and needs in the art that have not yet been fully solved by currently available techniques and processes.

Accordingly, the present invention has discloses the preparation of an improved quetiapine nanoemulsion formulation for intranasal drug delivery.

Further the instant invention is relates to stability, bioavailability and biodistribution study of the quetiapine nanoemulsion and compare it with the marketed formulation.

OBJECTS OF THE INVENTION

A primary objective of the present invention is to the preparation of quetiapine nanoemulsion for intranasal drug delivery.

Another objective of the present invention is to prepare the ternary phase diagrams using different ratios surfactant co-surfactant combination and study its effect on formation of nanoemulsion.

One more objective of the present invention is to compare in-vitro performance of prepared nanoemulsion with marketed formulation.

Other objective of the present invention is to carry out bioavailability and biodistribution study of the nanoemulsion and compare it with the marketed formulation.

Another objective of the present invention is to carry out stability studies of prepared formulation.

BRIEF DESCRIPTION OF FIGURES

These and other features, aspects, and advantages of the present invention will become better understood when the following detailed description is read with reference to the accompanying figures in which like characters represent like parts throughout the figures,

wherein:

Figure 1 to 9, illustrates different aspects indicating the processing steps and results analysis of the present invention**.**

DETAILED DESCRIPTION OF THE INVENTION

For the purpose of promoting an understanding of the principles of the invention, reference will now be made to the embodiment illustrated in the figures and specific language will be used to describe the same.

It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the illustrated system, and such further applications of the principles of the invention as illustrated therein being contemplated as would normally occur to one skilled in the art to which the invention relates.

It will be understood by those skilled in the art that the foregoing general description and the following detailed description are exemplary and explanatory of the invention and are not intended to be restrictive thereof.

Reference throughout this specification to “an aspect”, “another aspect” or similar language means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrase “in an embodiment”, “in another embodiment” and similar language throughout this specification may, but do not necessarily, all refer to the same embodiment.

The terms "comprises", "comprising", or any other variations thereof, are intended to cover a non-exclusive inclusion, such that a process or method that comprises a list of steps does not include only those steps but may include other steps not expressly listed or inherent to such process or method. Similarly, one or more devices or systems or elements or structures or components proceeded by "comprises... a" does not, without more constraints, preclude the existence of other devices or other systems or other elements or other structures or other components or additional devices or additional systems or additional elements or additional structures or additional components.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The system, methods, and examples provided herein are illustrative only and not intended to be limiting.

The terms “a” and “an” herein do not denote a limitation of quantity, but rather denote the presence of at least one of the referenced items.

The terms “having”, “comprising”, “including”, and variations thereof signify the presence of a component

The term “Extraction” is a separation process consisting of the separation of a substance from a matrix. Common examples include liquid-liquid extraction, and solid phase extraction. The distribution of a solute between two phases is an equilibrium condition described by partition theory. This is based on exactly how the analyst moves from the initial solvent into the extracting solvent.

The term “Drug delivery” refers to approaches, formulations, manufacturing techniques, storage systems, and technologies involved in transporting a pharmaceutical compound to its target site to achieve a desired therapeutic effect.

The term "Nanoemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules.

The instant invention discloses the preparation of nanoemulsion, wherein the drug is be dissolved in the lipophilic part of the Nanoemulsion i.e. oil and the water phases can be combined with surfactant and a cosurfactant is then added at slow rate with gradual stirring until the system is transparent. Ultrasonicator can be used to achieve the desired size range for dispersed globules. It is then being allowed to equilibrate. Gel may be prepared by adding a gelling agent to the above Nanoemulsion. Carbomers (cross linked polyacrylic acid polymers) are the most widely used gelling agent.

Another embodiment discloses the importance of the three important conditions:

- Surfactants must be carefully chosen so that an ultra-low interfacial tension can be attained at the oil / water interface which is a prime requirement to produce nanoemulsions.

- Concentration of surfactant must be high enough to provide the number of surfactant molecules needed to stabilize the microdroplets to be produced by an ultra-low interfacial tension.
- The interface must be flexible or fluid enough to promote the formation of Nanoemulsions.

In another embodiment, the instant invention discloses the construction of ternary phase diagrams, wherein the Pseudo-ternary phase diagrams of oil, water, and co-surfactant/surfactants mixtures are constructed at fixed cosurfactant/surfactant weight ratios. Phase diagrams are obtained by mixing of the ingredients, which shall be pre-weighed into glass vials and titrated with water and stirred well at room temperature. Formation of monophasic/biphasic system is confirmed by visual inspection. In case turbidity appears followed by a phase separation, the samples shall be considered as biphasic. In case monophasic, clear and transparent mixtures are visualized after stirring; the samples shall be marked as points in the phase diagram. The area covered by these points is considered as the Nanoemulsion region of existence

In addition to the above embodiment ,another embodiment describes the mechanism of action of Quetiapine, as with other drugs used to treat schizophrenia, is unknown. However, it is thought that Quetiapine's therapeutic activity in schizophrenia is mediated through a combination of dopamine type 2 (D2) and serotonin type 2 (5HT2) receptor antagonism. Although quetiapine is known to bind other receptors with similar affinity, only the dopamine D2 and serotonin 5HT2 receptor binding is responsible for quetiapine's therapeutic activity in schizophrenia

Working example

Example: 1

Solubility study of Quetiapine:

Prior to the preparation of Quetiapine nanoemulsion the solubility of quetiapine was determined in different oils such as Labrafil M 1944 CC, sesame oil, isopropyl myristate, lauroglycol 90, miglyol 840,capmul MCM. This is carried out by dissolving excess drug in fixed quantity of the oil. The mixture was kept under moderate magnetic stirring for 24 hours to reach the equilibrium. The sample was then centrifuged at 20000 rpm for 20 min. An a liquid of supernatant was collected and then diluted with ethanol. The content of Quetiapine in each sample was then determined by UV spectrophotometry.

Screening of surfactants for the preparation of nanoemulsion:

For the screening of surfactants Labrasol and Tween80 was selected. Tween80 is non-ionic surfactant and can be used in nasal preparation with no side effects on the nasal mucosa. Similarly Labrasol is widely used in nasal preparation and shows no irritant properties. Batches were prepared using above surfactants in different concentrations

Table.1 Screening of Surfactants

Batch code	Name of surfactant	Quantity of surfactant (%)	Quantity of oil (%)
T10	Tween 80	10	4%
L10	Labrasol	10	4%
T20	Tween 80	20	4%
L20	Labrasol	20	4%

Preparation of Ternary phase diagram:

The ternary phase diagrams for each surfactant–co-surfactant (Tween80:Emalex LWIS10, Tween80:PEG400, Tween80:Transcutol P) combination were made using different ratios of surfactant and co-surfactant i.e from 1:3 to 3:1. To prepare ternary phase diagrams, the surfactant and co-surfactant solution (Smix) was made and vortexed for 10min to mix properly. To that Capmul MCM (oil) was added. The ratio of oil to Smix was varied from 9:1 to 1:9 w/w. The water was added by titration method and the amount of water needed to turn the solution turbid was recorded as the end point.

Preparation of nanoemulsion of Quetiapine:

The oil in water nanoemulsion of Quetiapine was prepared by high sheer method using ultra probe sonicator. The different batches were prepared as per the ternary phase diagrams for each surfactant co-surfactant combination. The quantity of oil that requires dissolving the sufficient quantity of drug was chosen and for that the minimum amount of surfactant required was selected based on ternary phase diagram studies.

The nanoemulsion was prepared as per following steps:

1) Preparation of aqueous phase: Aqueous phase consists of surfactant and co-surfactant solution in water, prepared by moderate magnetic stirring.

2) Preaparation of oil phase: Oil phase prepared by dissolving Quetiapine (130mg) in Capmul MCM.

3) Preparation of nanoemulsion: The nanoemulsion was prepared by adding oil phase to aqueous phase under Sonication with ultra probe Sonicator. Sonication was done for 3 min 30 sec at 40˚C of probe temperature and 90% amplitude. Ice bath was used each time while

preparing batches. The emulsion was then filled in glass bottle and used for further study.

Blank nanoemulsion batches were prepared as follows based on the results of ternary phase diagrams:

Batches with Emalex LWIS 10 as co-surfactant:

Table.2 Blank nanoemulsion batches with emalex LWIS 10 as co-surfactant

Sr. No.	Batch No.	Tween80:Emalex LWIS10	Oil (%)	Water (%)	Smix(%)
1.	QE 1	1:1	10	57	33
2.	QE 2	1:1	15	52	33
3.	QE 3	2:1	20	46	34
4.	QE 4	3:1	10	60	30
5.	QE 5	3:1	20	45	35

Batches with PEG 400 as co surfactant:

Table.3 Blank nanoemulsion batches with PEG 400 as co-surfactant

Sr. No	Batch No.	Tween 80 : PEG 400	% of oil	% of water	% of Smix	Co-solvent
1.	QP 1	1:1	10	20	72	-
2.	QP 2	1:1	20	19	61	-
3.	QP 3	1:1	30	18	52	-
4.	QP 4	2:1	10	65	25	-
5.	QP 5	2:1	20	57	23	-
6.	QP 6	2:1	20	35	45	-
7.	QP 7	2:1	25	31	44	-
8.	QP 8	3:1	20	24	56	-
9.	QP 9	2:1	15	34	51	-
10.	QP 10	2:1	20	33	47	-
11.	QP 11	2:1	15	34	51	PG

Batches with Transcutol P as cosurfactant

Table.4 Blank nanoemulsion batches with TranscutolP as co-surfactant

Sr. No	Batch No.	Tween80: TranscutolP	Oil (%)	Water (%)	Smix (%)	Cosolvent
1.	QT 1	1:1	25	44	31	_
2.	QT 2	2:1	18	49	33	_
3.	QT 3	2:1	21	28	51	_
4.	QT 4	2:1	16	33	51	_
5.	QT 5	2:1	16	33	51	PEG 400

Working Example-2

Preliminary Experimental Studies:

Calibration curve for Quetiapine by UV visible spectrophotometer:

Calibration curve for Quetiapine was found to be linear over concentration range of 10 to 50 mcg/ml at λmax 288 nm in ethanol and 289nm in phosphate buffer by UV-Visible spectroscopy, with equation of line as y = 0.018x + 0.004, linearity constant R2 0.999. Plot of absorbance vs. concentration was plotted by using Microsoft excel (Fig1).

Melting Point:

Melting point of Quetiapine was found to be 174.50C.

Solubility study:

Before preparing nanoemulsion, solubility of Quetiapine was found out in various oil including Labrafil M 1944 CC, sesame oil, isopropyl myristate, lauroglycol 90, miglyol 840,capmul MCM. Solubility of Quetiapine was found be maximum in capmul MCM (table 5.1).

Table.5.1 Solubility of quetiapine in oils:

OIL	SOLUBILITY (mg/ml)
Labrafil M 1944 C	0.9 ± 0.087
Sesame oil	0
Isopropyl myristate(IPM)	0.54 ± 0.45
Lauroglycol 90	2.1 ± 0.04
Capmul MCM	6.3 ± 0.09
Miglyol 840	0.53 ± 0.23

Also the solubility of quetiapine was determined in solvents like PEG 400 and propylene glycol (PG) (table 5.2).

Table.5.2 Solubility of quetiapine in Solvent

COSOLVENT	SOLUBILITY(mg/ml)
PEG 400	7 .3 ± 0.6
Propylene glycol	6.9 ± 0.24

Screening of surfactant system:

Blank batches of nanoemulsion were formulated with Capmul MCM as oil phase. Tween 80 and Labrasol were used as surfactant system. It was observed that blank batches with 10% of Tween 80 and 10% of labrasol were not stable. The globule size of blank nanoemulsion with 20% of tween 80 and labrasol was found to be 676nm 1.3um reapectively. Hence Tween 80 was used as surfactant.

Ternary phase diagrams for Quetiapine:

From the solubility studies the combination of oil, surfactant and co-surfactant were selected. The ternary phase diagrams for each surfactant–cosurfactant combination were made by using different ratios of surfactant and co-surfactant i.e from 1:3 to 3:1. The readings obtained were entered into software “CHEMIX school 3_51” and the ternary phase diagrams were obtained. The readings obtained by ternary study were converted to the percentages before entering values in the software. The shaded area in the ternary phase diagram is the probable emulsion area. The sufficient quantity of oil required to dissolve drug was selected and the minimum quantity of Smix in the shaded area was selected to form emulsion. The batches were prepared according to selected quanitity of oil, Smix and water.

From the ternary phase diagrams (Fig.2 to 4) it is observed that the emulsion regions (shaded region) in case of Emalex LWIS 10 and Tween 80 are smaller as compared to the emulsion region obtained with Transcutol P as co-surfactant. That may be due to the HLB value of Transcutol P i.e 4.5 which is much closer to the RHLB value of Capmul MCM 8.3. So Transcutol P can form emulsion with higher percentage of oil.

Working Example-3

FORMULATION STUDY:

Preparation of Quetiapine nanoemulsion (QNE):

The oil in water nanoemulsion of quetiapine was prepared by high sheer method using ultra probe sonicator. The different batches were prepared as per the ternary phase diagrams for each surfactant co-surfactant (Smix) combination. The prepared nanoemulsion observed for consistency and any phase separation. The batches which showed phase separation (unstable) and batches which were too viscous were rejected.

Blank batches with Emalex LWIS10 as co-surfactant

Table.6.1 Blank batches with Emalex LWIS 10

Sr. No	Batch No.	Tween80:Emalex LWIS10	Oil (%)	Water (%)	Smix (%)	Result
1)	QE 1	1:1	10	57	33	Not stable
2)	QE 2	1:1	15	52	33	Not stable
3)	QE 3	2:1	20	46	34	Not stable
4)	QE 4	3:1	10	60	30	Not stable
5)	QE 5	3:1	20	45	35	Not stable

Blank batches with PEG 400 as cosurfactant

Table.6.2 Blank batches with PEG 400

Sr. No	Batch No.	Tween 80 : PEG 400	Oil (%)	Water (%)	Smix (%)	Cosolvent	Result
1)	QP 1	1:1	10	20	72	-	Viscous
2)	QP 2	1:1	20	19	61	-	Viscous
3)	QP 3	1:1	30	18	52	-	Viscous
4)	QP 4	2:1	10	65	25	-	Not stable
5)	QP 5	2:1	20	57	23	-	Not stable
6)	QP 6	2:1	20	35	45	-	Large size
7)	QP 7	2:1	25	31	44	-	Large size
8)	QP 8	3:1	20	24	56	-	Stable
9)	QP 9	2:1	15	34	51	-	Stable
10)	QP 10	2:1	20	33	47	-	Large size
11)	QP 11	2:1	15	34	51	PG	Stable

Blank batches with Transcutol P as cosurfactant

Table.6.3 Blank batches with Trnascutol P

Sr. No	Batch No.	Tween80: TranscutolP	Oil (%)	Water (%)	Smix (%)	Cosolvent	Result
1)	QT 1	1:1	25	44	31	_	Not stable
2)	QT 2	2:1	18	49	33	_	Not stable
3)	QT 3	2:1	21	28	51	_	Stable
4)	QT 4	2:1	16	33	51	_	Stable
5)	QT 5	2:1	16	33	51	PEG 400	Stable

All batches with Emalex LWIS 10 as co-surfactant were not stable. From ternary phase diagrams, the percentage of Smix in case of Emalex LWIS 10 that can be used to prepare nanoemulsion is very low. That much percentage of Smix seems to be not able to stabilize the system. Hence that can be the reason why all batches with Emalex LWIS 10 failed.

Batches QP1, QP2 and QP3 were too viscous. Batches QP2, QP3 have less amount of water than oil so they were very viscous. Batches QP7, QP8, QP10 have higher amount of oil that cannot be stabilized by used percentage of Smix, so the batches were not stable. Batches QP8, QP9 and QP11 are prepared by using higher amount of Smix, hence these batches were stable.

Batches QT1 and QT2 were not stable and nanoemulsion broke immediately after the preparation. Reason may be the same as explained above, the higher percentage of oil that cannot be stabilized by the used percentage of Smix.

Among all the blank batches, QP8, QP9, QP11, QT3, QT4 and QT5 found to be stable. So drug loaded batches were prepared and further analysis was carried out.

Evaluation and characterization of prepared nanoemulsion:

Globule (droplet) Size Determination:

The main feature common to all emulsions is their strict droplet size, which must be in a nanometer range. Therefore, particle size analysis was performed to confirm whether the resultant emulsions were indeed nanoemulsions. Globule size of each batch was evaluated by laser diffractometer (Malvern Hydro 2000 SM). After formulation batches were stored at room temperature as well as at 40˚C and their particle size were measured after specific period of time.

Table.7 Globule size analysis of drug loaded batch:

Sr. No.	Batch No.	Globule Size(nm)
1)	QP 8	339 ± 5
2)	QP 9	289 ± 0.7
3)	QP 11	144 ± 0.5
4)	QT 3	464 ± 0.75
5)	QT 4	204 ± 0.63
6)	QT 5	151 ± 0.65

Drug loaded batches of Quetiapine nanoemulsion showed globule size in nano range. The adequate percentage of Smix used in drug loaded batches helped to reduce the size of nanoemulsion to nano range. Batches QP11 and QT5 were prepared using cosolvents (propylene glycol and PEG400 respectively). These batches showed the smaller globule size and also showed greater stability. No sedimentation and no change in the appearance were observed. Hence these two batches were selected for further studies and evaluation.

Zeta (ζ) potential determination:

Zeta potential is the main indicator of emulsion stability.[1] As all the excipients used were non-ionic in nature, low zeta potential values could be attributed to the drug molecule.

Table.8 zeta potential of the nanoemulsions

Sr. No.	Batch	Zeta Potential [mV]
1.	QP 11	-8.131
2.	QT 5	-5.69

Drug content of the nanoemulsion:

Drug content in the prepared nanoemulsion formulations was determined using UV-Visible spectrophotometer (UV, 1601, 220X Shimadzu, Japan) at λmax 288.0 nm using ethanol as blank. The 1ml of the sample (nanoemulsion) was diluted with 10 ml of ethanol and the absorbance was recorded. Drug content of the batches QP11 and QT5 was found to be 91% and 89% respectively.

Transmission Electron Microscopy (TEM):

The surface appearance and shape of Quetiapine nanoemulsion was analyzed by taking TEM photographs. TEM of formulations reveals that the nanoemulsion particles are spherical with homogeneity. The size of the nanoemulsion globules is about 139.5 nm(Fig 5)which is much similar to that determined by the Malvern Hydro 2000 SM particle size analyzer.

Stability study of prepared nanoemulsions:

Table.9 Stability study of the prepared nanoemulsion

Batch Code:	Globule size (nm)										
	Initial	7 days		15days		1month		2 months		3 months	
		25°C	40°C	25°C	40°C	25°C	40°C	25°C	40°C	25°C	40°C
QP8	339	342	350	341	349	358	360	375	368	380	392
QP9	289	290	296	271	285	292	305	209	223	208	234
QP11	144	145	146	144	144	148	150	144	153	148	150
QT3	464	468	467	504	519	544	532	547	559	550	553
QT4	204	268	236	252	229	251	212	253	258	253	261
QT 5	151	153	148	153	155	156	162	159	152	162	210

From stability studies, batches QT3 and QT4 shows drug sedimentation. Batches QP11 and QT5 were prepared using cosolvents (propylene glycol and PEG400 respectively). So these batches showed the smaller globule size and also showed greater stability. Batches QT5 and QP11 did not show much change in globule size over a period of 3 months and were stable as compared to other batches in terms of globule size. No sedimentation and no change in the appearance observed. These two batches were selected for further studies and evaluation.

In-Vitro Drug Release Studies:

The release of Quetiapine from nanoemulsions and the marketed formulation was determined using the dialysis technique in phosphate buffer pH 6.4

Table.10.1 R2 values for zero order drug release:

Batch	R^2
MKTD	0.795
QT4	0.890
QT5	0.891
QP 11	0.934

Table.10.2 R2 values for 1st order drug release

Batch	R^2
MKTD	0.882
QT4	0.952
QT5	0.959
QP 11	0.98

From figure 6, it is observed that the drug release from QT4 is lesser than the drug release from batch QT5. This may due to the cosolvent system used in QT5. About 80% release observed for each nanoemulsion batch and also for the marketed formulation. Release kinetics seems to be a square root time dependent. R2 values of the drug release are higher for the higuchi maodel i.e 0.882, 0.952, 0.959 and 0.98 for Marketed formulation, batch QT4, QT5 and QP11 respectively, than the R2 values for zero order release. Kinetics, as well as the prolonged drug release observed in vitro can be explained by the fact that quetiapine diffusion from the oily core and interface is hindered by the aqueous medium, which acts as a barrier to drug transport due to its very low solubility in water.

In-vivo studies:

High pressure liquid chromatography (HPLC)

The HPLC method proposed provides a simple procedure for the determination of Quetiapine (QTP) in biological samples. A straight forward and accurate HPLC method to determine Quetiapine in rat plasma and brain tissue was initially developed. Chromatographic condition, based on the isocratic separation, gave a good profile in plasma using a reverse phase C18 column. Ammonium acetate (20mm) : Acetonitrile 50:50 v/v was used as a mobile phase. The pH of the mobile phase was adjusted to 6.4 with the help of acetic acid. The Quetiapine detection was carried out at 235 nm, since at this wavelength maximum response is achieved along with the absence of endogenous interfering peaks occurring from blank matrix. The samples of Quetiapine were successfully extracted from interfering plasma using methanol (extraction solvent) with excellent recoveries. In the present study, Olanzapine (OLZ) was selected as internal standard because of its similar physico-chemical characteristics as that of Quetiapine. Both drugs possess poor aqueous solubility and a benzothiazepine moiety in their structure.

Extraction efficiency

Various extraction procedures including protein precipitation methods and liquid–liquid extraction was investigated. Direct protein precipitation with acetonitrile gave low recovery with high background noise. For liquid–liquid extraction procedure selection of suitable solvent is a critical parameter. Ideally, the selected solvent should be compatible, immiscible with water, stable enough over the extraction time and have high solubility for the target analyte. A series of organic solvents and their mixtures of varying polarity [viz. acetonitrile, methanol, chloroform, ethyl acetate, acetonitrile : methanol (1:1), diethyl ether and dichlorometane : diethyl ether (1:1)] were evaluated for extraction of quetiapine from plasma and brain. It was observed that methanol effectively eliminates the interfering material and gave the best recovery for Quetiapine**.**

Bioavailability and biodistribution studies:

Bioavailability studies performed on the nanoemulsion administered intranasally and the bioavailability was compared with that of the marketed formulation. The quetiapine concentration in brain following the intranasal (i.n.) administration of quetiapine nanoemulsion was found to be significantly higher at all the time points compared to both oral route and intranasal route (fig,9).

Also biodistribution studies of formulations following intravenous administration and intranasal administrations on male wistar rats were performed and the drug concentration was estimated at different intervals upto 8 h. The quetiapine concentration in brain following the intranasal (i.n.) administration of quetiapine nanoemulsion was found to be significantly higher compared to both oral and intravenous route.

Table.11 Comparison of kinetic parameters obtained by each given route

Route		Cmax(%/g)	Tmax(h)	AUC_{0-480} (h%/g)
Oral	Brain	0.35	3	0.294
	Blood	0.72	2	0.447
IN	Brain	0.485	1	0.35
	Blood	9.2	2	0.74
IV	Brain	0.256	3	0.154
	Blood	16	0.25	1.036

The lower Tmax values for brain (1 h) when compared to blood (2 h) may also be attributed

to preferential nose to brain transport following intranasal administration. When the Cmax and AUC of brain concentration of quetiapine nanoemulsion (i.n), intravenous and oral were compared, the Cmax (0.485%/g) and AUC (0.35 h %/g) of quetiapine nanoemulsion were found to be significantly higher.

DTP% (direct nose to brain transport) and DTE% (drug targeting efficiency) were calculated by formulae given below, using tissue/organ distribution data following intranasal and intravenous administrations and are recorded in Table 12.

$$DTE\% = \left[\frac{(AUC_{brain}/AUC_{blood})_{i.n.}}{(AUC_{brain}/AUC_{blood})_{i.v.}}\right] \times 100$$

$$DTP\% = \left[\frac{B_{i.n.} - B_x}{B_{i.n.}}\right] \times 100$$

$$\text{where } B_x = (B_{i.v.}/P_{i.v.}) \times P_{i.n.},$$

Table 12 : DTE(%) and DTP(%) values:

DTE(%)	DTP(%)
321	68

The DTP% represents the percentage of drug directly transported to the brain via the olfactory pathway. The higher DTE% and DTP% suggest that quetiapine naoemulsion has better brain targeting efficiency mainly because of substantial DTP via the olfactory region of the nasal cavity

We claim,

1. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonication comprising the steps of:

a. preparation of aqueous phase, wherein the aqueous phase consists of surfactant and co-surfactant solution in water, prepared by moderate magnetic stirring;
b. preparation of oil phase, wherein the Oil phase prepared by dissolving Quetiapine (130mg) in Capmul MCM;
c. preparation of nanoemulsion, wherein the nanoemulsion prepared by adding oil phase to aqueous phase under Sonication with ultra-probe Sonicator for 3 min 30 sec at 40˚C of probe temperature and 90% amplitude along with the ice bath for each batches.

2. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonicator as claimed in claim 1, where in different batches of nanoemulsion were prepared as per the ternary phase diagrams for each surfactant co-surfactant (Smix) combination using different ratios of surfactant and co-surfactant i.e from 1:3 to 3:1.

3. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonicator as claimed in claim 1, where in nanoemulsion formulations QP8, QP9, QP11, QT3, QT4 and QT5 are found to be stable.

4. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonicator as claimed in claim 1, where in nanofromulation Batches QP11 and QT5 (were prepared using cosolvents propylene glycol and PEG400 respectively) showed the smaller globule size and greater stability with zeta potential -8.131 and -5.69 mv respectively.

5. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonicator as claimed in claim 1, where in the batches QP11 and QT5 shows high percentage of drug content i.e 91% and 89% respectively.

6. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonicator as claimed in claim 1, where in the In-Vitro Drug Release Studies exhibits the

formulation QP11 and QT5 having R2 values of the drug release are higher for the higuchi maodel i.e 0.98 and 0.959 respectively.

7. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonicator as claimed in claim 1, wherein the nanoemulsion formulations of Quetiapine are used for intranasal drug delivery.

8. The process oil in water nanoemulsion of Quetiapine by high sheer method using ultra probe sonicator as claimed in claim 1, wherein higher DTE% and DTP% suggest that quetiapine naoemulsion has better brain targeting efficiency mainly because of substantial DTP via the olfactory region of the nasal cavity.

Date…04/09/2022 Signature…….

Abstract

An Improved Quetiapine Nanoemulsion Formulation and Its Therapeutic Application Thereof

The preparation of quetiapine nanoemulsion for intranasal drug delivery by the ternary phase diagrams using different ratios surfactant co-surfactant combination and study its effect on formation of nanoemulsion. Further the present invention is to compare in-vitro performance of prepared nanoemulsion with marketed formulation and to carry out bioavailability and biodistribution study of the nanoemulsion and compare it with the marketed formulation.