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Supplementary Materials: Quality by Design Assisted Optimization and Risk Assessment of Black Cohosh Loaded Ethosomal Gel for Menopause: Investigating Different Formulation and Process Variables

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Table S1. Possible failure modes affected by altering CMAs and CPPs derived from CQAs. Here, each CQAs has been examined with respect to all the related CMAs and CPPs. It further discusses how these CMAs and CPPs affect the CQAs resulting in the failure of the formulation.

CQAs	Related to CMAs	Related to CPPs	Failure Mode
Particle size	Change in raw material particle sizes	• Speed of mixing • Time of mixing • Injection rate	Changes in content uniformity, drug release & dermal distribution of the drug
PDI	Variation in particle sizes distribution of raw materials	• Speed of mixing • Time of mixing • Injection rate	Changes in content uniformity, drug release & dermal distribution of the drug
Ethanol concentration	Change in proportion of ethanol & water	• Process temperature • High duration of mixing	Changes in formulation microstructure, particle size, skin retention, permeation & impact on sensorial attributes of the product
Lipid concentration	Change in proportion of lipid & water	Process temperature	Alteration in size, PDI & entrapment
Surfactant concentration	Change in proportion of surfactant & other raw materials	Specific concentration is desired	Decrease the stability & alters absorption
pH	Amount of TEA in the formulation	Consistent addition of TEA with optimized stirring	Alters ionic strength
Temperature	-	Consistent temperature	Non-uniform size & PDI
Solubility	Change in raw material	Temperature	Precipitation & phase separation
Viscosity (shear stress & shear rate)	• Viscosity of liquid/semisolid raw materials • Concentration of polymer	Speed of mixing Time of mixing	Changes in skin retention of the formulation & drug penetration through the skin Changing in patient acceptability/compliance, Impact on sensorial attributes of the product
Content uniformity	-	• Speed of mixing • Time of mixing	Dose Variability
Drug entrapment	Changes in lipid & ethanol concentration	• Speed of mixing • Time of mixing	Affecting drug delivery, dermal dissemination & dosing frequency

Drug release	Alteration in lipid & ethanol concentration	• Rotation speed • Temperature of medium	Affecting drug delivery, dermal dissemination & formulation failure
Needle type	Alteration in needle size	• Speed of mixing • Time of mixing	Affecting the particle size & PDI
Injection rate	Defective plunger	• Speed of injection(plunger pressure) • Speed of mixing • Time of mixing	Inhomogeneous entrapment, size & multiplied PDI
Precipitation/aggregation	Dependent on the type of emulsifier, gelling agent or volatiles	• The order of addition • High duration of mixing	Influence on API partitioning within the formulation, Amount of drug permeating through the skin

Table S2. FMEA of the material attributes and process parameters involved in the preparation of the formulation along with their failure effects, cause and controls in quantifying risk. The attribute of failure modes of critical material attributes and critical process parameters are classified into high, medium and low risks. These risks were quantified basis on their failure effect, potential cause and control established.

Check Points/Inputs	Failure Mode	Failure Effects	S	Potential Cause	O	Controls established	D	RPN	Risk
				CPP					
Particle size	Larger particle size	Changes in dose uniformity, drug release & dermal distribution of the drug	8	Alterations in the concentration of ethanol, lipid, temperature & stirring speed are key factors affecting the size	8	The measured amount of ethanol & lipid will be a decisive effect with control temperature & stirring speed	7	448	High
PDI	Non Uniformity	Changes in dose uniformity, drug release & dermal distribution of the drug	5	Alterations in the concentration of ethanol, lipid, temperature & stirring speed are key factors affecting the size	6	The measured amount of ethanol & lipid will be a decisive effect with control temperature & stirring speed	5	150	Medium
Drug entrapment	Affects drug release	Varying minimum effective concentration	8	Solubility, amount of lipid & ethanol	8	Optimized amount of ethanol & lipid	6	384	High
Drug release	Changes in effectiveness /pharmacological action	Formulation failure	9	Improper drug entrapment & drug loading	6	Appropriate drug entrapment & loading	5	270	Medium
Content uniformity	Changes in dose uniformity	Variable dosing & drug release from the formulation	5	Inadequate solubility, & high PDI	5	Low PDI with suitable excipients	4	100	Low
Ethanol concentration	Change in particle size and PDI	Changes in the size of the formulation, PDI, drug	9	Contributes majorly to drug & lipid solubility. Further, it	8	Optimized quantity of ethanol is critical for parameters like	9	648	High

		entrapment, skin retention and permeation of the active ingredient		acts as a penetration enhancer hence, alteration of its concentration has a marked effect on drug penetration & size of the particles		particle size, PDI, drug entrapment, drug penetration & drug loading			
Lipid concentration	Inadequate size, drug entrapment & drug loading capacity	Changes in the size of the formulation, drug entrapment & drug loading	9	Lipid concentration contributes majorly to formulation thereby affecting size, drug entrapment & loading capacity	8	An accurate quantity of lipid concentration is crucial for related parameters	8	576	High
Surfactant concentration	Instability	Alteration in size, PDI & stability of the formulation	2	Contributes majorly to formulation stability	3	Optimized concentration of the surfactant concentration is essential for formulation stability	3	18	Low
Solubility	Variation in drug content, drug loading, particle size & PDI	Differing amounts of drug concentration thereby resulting in assorted drug loading, particle size and PDI	7	Solubility is affected by solvents & the amount meant for solubilising the active ingredient & excipients to uniformity. Improper solvents mean non-uniform solubility	6	Proper selection of excipients	4	168	Medium
Log P	Inadequate permeation	Changes in skin permeation & skin physiology	3	Inherent properties of the active ingredient	3		3	27	Low
Viscosity, shear stress and shear rate	▪ Changes in skin adhesion & permeation ▪ Extrusion from the container	▪ Alteration in skin adhesion of the formulation & followed by drug penetration through the skin ▪ Impact on sensorial attributes of the product ▪ Affect patient compliance	5	Inadequate gelling agent or dilution	6	Optimized amount of gelling agent	3	90	Low
CMA									
pH	Skin irritation	Variation in drug release & skin irritation	2	Improper mass ratio leads to incomplete conversion	3	Definite PH is required to avoid skin irritation & drug release	2	12	Low
Temperature	Affects lipid solubility	Alteration in size & PDI of the	1	At lower temperature size	2	Temperature is heavily monitored &	2	4	Low

		formulation. Hence drug entrapment, loading & drug release		and PDI of the formulation increase		fixed to the desired level			
Needle type	Variation in size of the formulation & PDI	Improper particle size & PDI hence causing variation in dose uniformity	2	Variation in needle type	3	Proper selection of needle	2	12	Low
Injection rate/ Flow rate	Changes in size of the formulation & PDI	Improper particle size & PDI hence causing variation in dose uniformity & coagulation of particles	3	Non-uniform flow rate	3	Optimized flow rate	2	18	Low
Stirring speed	Alteration in size & PDI of the formulation	Improper particle size & PDI hence causing variation in dose uniformity & coagulation of particles further, it affect the drug entrapment & loading	7	Improper speed of stirring	6	Maintaining proper stirring speed	5	210	Medium
Stirring time	Alteration in size & PDI of the formulation	Improper particle size & PDI hence causing variation in dose uniformity & coagulation of particles further, it affects the drug entrapment & loading	3	Improper speed of stirring	4	Maintaining proper stirring speed	3	36	Low

Table S3. Scoring table designed by the formulator considering the severity, probability of occurrence and probability of detectability of failure mode of the critical quality attributes associated with the formulation. The score was devised in the range of 1-10.

Score	Severity	Probability of Occurrence	Probability of Detectability
1	No Effect	No documented probability of failures	Controls in place to detect the fault
2-4	Minor-Limited part is affected	Low probability of failures to take place	Low probability that the fault will get undetected
5-7	Medium	Occasional probability of failures to take place and will have moderate impact on associated parameters	Moderate probability that the fault will get undetected
8-10	High	High probability of failures to take place and will have a high impact on associated parameters	High probability that the fault will get undetected

Table S4. Risk-level rating criteria. The risks were denoted as low, medium and high according to the risk priority number. It gives a realistic picture with a correlation of all variables related to process and materials with scrupulous importance to CQAs resulting in precise quantification of threat during the formulation process.

Risk priority number (RPN)	Risks level	Rationale
1-100	Low	The process is robust or controls are in place to handle any change
101-350	Medium	Process parameters & associated controls are comparatively stout & are capable enough to withstand moderate outcome
351-1000	High	Any alteration to process parameters/attributes will have serious outcome

Table S5. Formulation trials for initial risk assessment. It describes how the concentration of lipid, ethanol, and surfactant; temperature; time of stirring; the speed of stirring affect the particle size and PDI of the formulation.

Formulation code	Parameter	Particle size (nm)	PDI
Concentration of Ethanol			
N1	30 %	405	0.52
N2	40 %	188	0.30
N3	45 %	85	0.21
Concentration of Lipid (Lipoid S 100)			
N4	2 %	83	0.18
N5	3 %	235	0.35
N6	4 %	445	0.44
Concentration of surfactant (Tween 80)			
N7	1%	84	0.16
N8	2%	118	0.26
Temperature			
N9	25	410	0.41
N10	30	84	0.15
N11	35	82	0.19
Stirring time			
N12	5 minutes	312	0.4
N13	10 minutes	82	0.13
N14	15 minutes	89	0.15
Stirring speed			
N15	1900 rpm	225	0.2
N16	2200 rpm	85	0.15
N17	2300	81	0.16

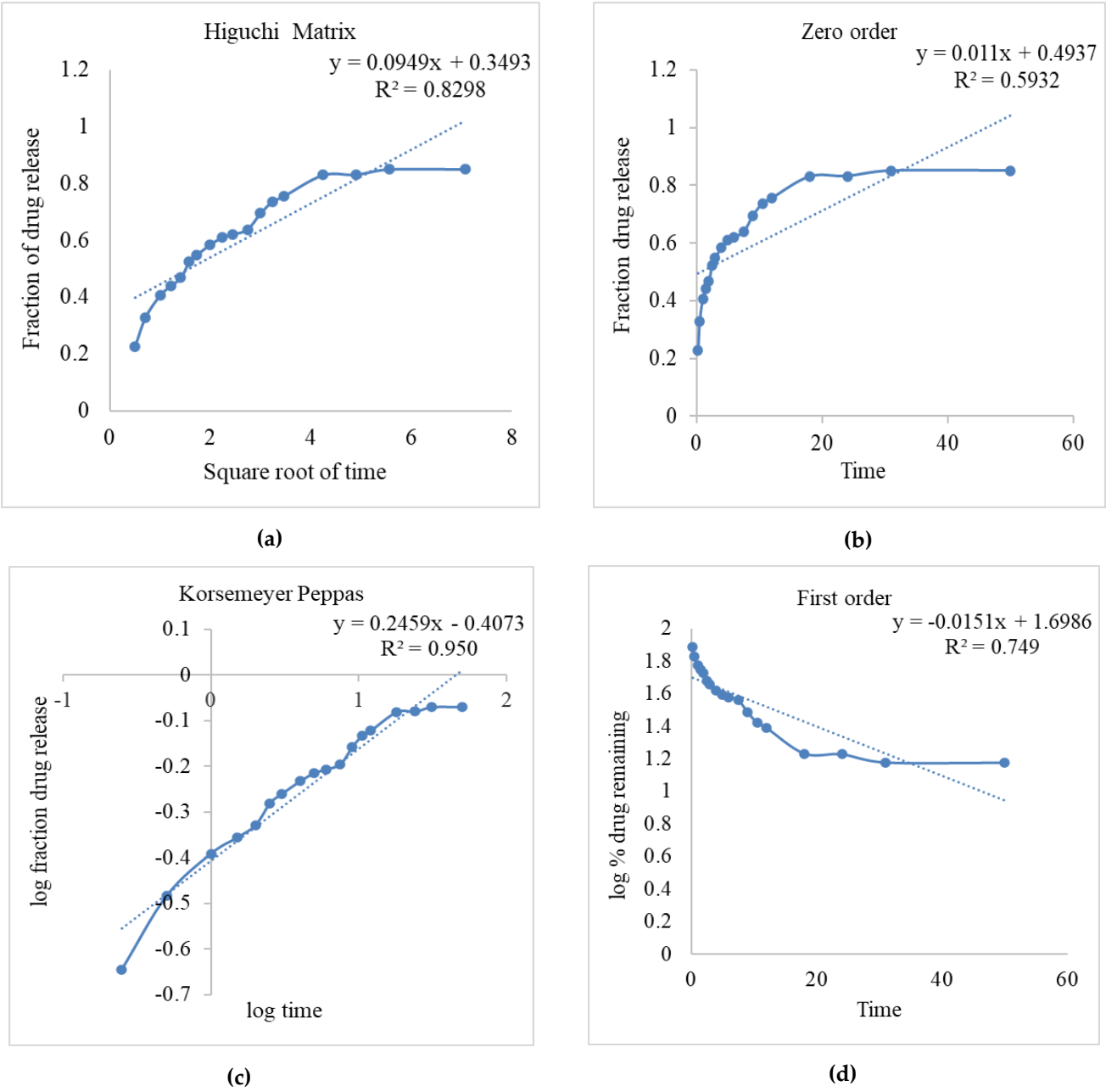

Figure S1. Different release kinetic modelling for ethosomal gel to support the release analysis. (**a**) Higuchi matrix (**b**) Zero order kinetics (**c**) Korsemeyer Peppas (**d**) First order kinetics.