

Table of contents

1	Introduction	1
2	Literature Review	9
2.1	Breast cancer	9
2.1.1	Breast cancer statistics	9
2.1.2	Challenges	11
2.1.3	Causes Etiology	11
2.2	Breast cancer treatments	12
2.2.1	Chemotherapy	13
2.2.2	Drugs approved by FDA	14
2.3	Drug Combinations Used in Breast Cancer	15
2.3.1	Need of drug combinations	15
2.3.2	Anticancer drug combination strategies	16
2.3.3	Methodology	18
2.3.4	Limitations associated with drug combination studies	19
2.3.5	Research envisaged	20
2.4	Nanomedicine	22
2.4.1	Solid lipid nanoparticles (SLNs)	22
2.4.2	Aqueous core nanocapsules(ACN)	23
2.5	Drug and excipient profile	23

2.5.1	Vinorelbine bitartrate	23
2.5.2	Resveratrol	31
2.5.3	Glyceryl monooleate (GMO)	35
2.5.4	Glyceryl monostearate (GMS)	37
2.5.5	Poly (lactic-co glycolic acid), PLGA	39
2.5.6	TPGS d- α -tocopheryl polyethylene glycol 1000 succinate	42
2.5.7	Poloxamer-188 (PL-188)	45
3	Objectives and Plan of Work	48
3.1	Objectives	48
3.2	Detailed research plan	49
4	Materials and Methods	51
4.1	Materials	51
4.2	HPLC analytical method development for simultaneous estimation of VRL and RES	54
4.2.1	HPLC conditions	54
4.2.2	Selection of mobile phase	54
4.2.3	Standard stock solution preparation	54
4.2.4	Identification of λ_{max} for VRL and RES	55
4.2.5	Sample preparation	55
4.2.6	Calibration curve preparation	55

4.2.7	Method validation	56
4.3	Formulation development	59
4.3.1	Preliminary screening of polymers and lipid	59
4.3.2	Solid Lipid Nanoparticles	59
4.3.3	Aqueous core nanocapsules (ACNs)	62
4.3.4	Dual drug loaded aqueous core nanocapsules (dd-ACNs)	64
4.4	Characterization of nano-formulations	67
4.4.1	Particle size, polydispersity index (PDI) and zeta potential	67
4.4.2	Total drug content (TDC) and encapsulation efficiency (EE)	68
4.4.3	Morphology of nano-formulations	69
4.4.4	FTIR	69
4.4.5	DSC analysis	70
4.4.6	In-vitro drug release studies	70
4.4.7	Stability Studies	71
4.5	Safety for intravenous administration	72
4.5.1	Evaluation of haemolysis.	72
4.5.2	Platelet aggregation	73
4.6	<i>In-vitro</i> anticancer activity	73
4.7	Toxicity studies	74
4.7.1	Animals	74

4.7.2	Experimental protocol	74
4.8	<i>In-vivo Anticancer efficacy</i>	75
4.8.1	Animals	75
4.8.2	Experimental protocol	75
4.9	Statistical analysis	77
5	Results and Discussions	78
5.1	HPLC analytical method development for simultaneous estimation of VRL and RES	78
5.1.1	Identification of λ_{max} for VRL and RES	78
5.1.2	Calibration curve	78
5.1.3	Method validation	81
5.2	Preliminary screening	84
5.3	Solid lipid nanoparticles (SLNs)	84
5.3.1	Experimental design	84
5.3.2	Determination of optimal conditions for preparation of TPGS-VRL-SLNs and PL-VRL-SLNs	90
5.3.3	Characterization of VRL-SLNs	91
5.3.4	<i>In-vitro</i> cytotoxicity studies	98
5.3.5	Safety for intravenous administration	101
5.4	Aqueous core nanocapsules (ACNs)	102
5.4.1	Experimental design	102

5.4.2	Characterization of GMS-VRL-ACNs	109
5.4.3	<i>In-vitro</i> cytotoxicity	114
5.4.4	Safety for intravenous administration	117
5.5	Dual drug loaded ACNs (dd-ACNs)	120
5.5.1	Determination of synergistic ratio of VRL and RES	120
5.5.2	Characterization of ACNs	123
5.5.3	Cytotoxicity Studies against MCF-7 breast cancer cell line	128
5.6	Comparative study of all formulations	130
5.7	<i>In-vivo</i> studies	132
5.8	<i>In-vivo</i> anticancer studies	134
6	Conclusion	137
7	References	139
8	Addendum	
8.1	Nanomedicine	149
8.1.1	Polymeric nanoparticles	149
8.1.2	Liposomal nanoparticles	149
8.1.3	Protein-drug conjugated nanoparticles	150
8.1.4	Dendrimeric nanoparticles	152

8.1.5 Micellar nanoparticles

15

8.1.6 Other nanoparticle platforms

15

8.2 Research Envisaged

152

8.3 Plan of work- flow chart

157