Original Research Article

Analytical tools used for characterization and development of Minitablets:

A Verapamil Hydrochloride Case Study

1. Abstract:

The various analytical techniques were used at different stages of formulation development to assess the interactions and quality throughout the lifecycle of the verapamil hydrochloride minitablets (VHMT). At initial stage of development studies, pre-formulation analytical techniques like FTIR and DSC used to evaluate the interactions between the drug substance with different inactive ingredients and physicochemical properties of drug substance, which provided the groundwork for the development of robust formulation. As a part of physicochemical properties, the solubility data of verapamil HCl exhibited that pH dependent solubility throughout the physiological buffer media from pH 1.2 – 6.8, as pH of media increase solubility decrease due to the weak basic nature (pKa) of Verapamil HCl. To improve the solubility of VH, Fumaric acid was included in the formulation. The analytical data of FTIR and DSC showed that no chemical interaction with selected excipients. The formulation analytical quantitative techniques such as a simple stability indicating HPLC assay procedure has been developed and validated for verapamil minitablets life cycle (initial and stability samples). The analytical data of stability samples of VHMT showed stable up to 3M at 40°C. The pre-formulation data at initial development stage and the stability data of final product evidences that the final drug product was developed with desired release characteristics without any instability issues. Overall, the combined use of pre-formulation and formulation analytical techniques helped to identify the defects at early stage of

development and overcome those shortcomings by appropriate scientific approach, which significantly minimized the formulation failure at later stage.

KEY WORDS: Multiarticulates, pulsatile release minitablets, verapamil hydrochloride, chronotherapeutic drug delivery, fumaric acid and ethyl cellulose, preformulation, formulation analytical techniques.

2. Introduction:

Verapamil hydrochloride (VH) is a calcium channel blocker used for the treatment of high blood pressure, heart arrhythmias and angina by relaxing the blood vessels and minimizing the pressure on heart. It also increases the supply of blood and oxygen to the heart and slows electrical activity in the heart to control the heart rate [1]. Therefore, verapamil is considered as one of the drugs of choice for the cardiovascular therapies. In general, most of the cardiovascular dosage regimens are optimized based on circadian rhythm pattern of blood pressure with morning rise and decline during night [2]. Due to these natural fluctuations in blood pressure, the antihypertensives will be prescribed as a combination of drugs or multiple doses per day for effective control of blood pressure [3], which leads to the poor drug adherence by causing the pill burden on patients [4]. Chrono therapeutics, a pulsed release system developed to enhance therapeutic efficiency and patient compliance by reducing the pill burden. These pulsed system dosage forms release the drug at desired rate at selected time to mimic the circadian rhythms [5]. However, the development of these complex therapeutic dosage (pulsed release system) forms is very challenging, and many factors need to be optimized prior to the in-vivo study to minimize the failure of release characteristics during in-vivo pharmacokinetic study. Therefore, during formulation development, a series of quality control checks steps will be designed and executed to identify the problem at early

development. Numerous analytical techniques are available to help the formulation development team in identifying these shortcomings at early development stage.

These analytical techniques are classified as pre-formulation and formulation methods. The pre-formulation methods [6] are helpful to characterize the physiochemical properties of drug substance like solubility, rheological properties, pKa, particle size, crystalline nature, excipient compatibility (by Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry) etc. Whereas the formulation methods (like spectroscopic methods) [7] are helpful to assess impurities, percent purity and in-vitro dissolution characteristics of the drug product. Therefore, the use of appropriate pre-formulation and formulation analytical methods leads to development of dosage form with desired drug release characteristics with a minimal in-vivo study failure risk [8], which saves significant time/resources and cost. In the current research, the authors discussed the various pre-formulation and formulation analytical methods employed during optimization of the pulse release dosage form of verapamil with emphasize on how the authors identified the deficiencies at early on and changed the optimized conditions which minimized the formulation failure at later stage.

3. Materials:

Verapamil hydrochloride (VH), an anti-hypertensive is obtained as a gift sample from Piramal Healthcare Limited, Medak, India. Microcrystalline cellulose (MCC) of two grades of Avicel PH 101 and PH 102, used as diluents; polyvinylpyrolidone (PVP K30) used as binder, polyethylene glycol (PEG400) used as plasticizer; ethylcellulose (EC100cps), used as controlled release polymer; magnesium stearate, used as lubricant; fumaric acid, used as pH modifier and isopropyl alcohol, used as granulating liquid were purchased from S.D. Fine Chem. Pvt. Ltd., Chennai. All the excipients used in the study were of pharmaceutical grade. HPLC grade dichloromethane were purchased from Sigma Chemical Industries, Hyderabad.

4. Experimental:

4.1 Preformulation studies:

Pre-formulation studies were used to evaluate the physicochemical characteristics of verapamil HCl and to identify appropriate compatible excipients and the conditions under which the drug substance is stable. The various parameters were monitored during this stage which includes melting point determination, solubility evaluation and excipient compatibility verification studies [9,10].

4.1.1 Melting point determination (Drug identification test)

The melting point determination was conducted by two different techniques, capillary fusion method and DSC method.

Capillary fusion method: A small quantity of powder was placed into a fusion tube. The tube was placed in the melting point determining apparatus. The temperature of the apparatus was gradually increased and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

DSC method: The DSC curves were obtained on a TA Instruments Calorimeter; model DSC Q20. A small quantity of powder about 2 ± 0.1 mg was placed in an aluminium crucible under nitrogen atmosphere, at the flow of 50 mL min-1 and applied the temperature with ramp rate of 5 $^{\circ}$C/min. Data was analyzed using the software TA Instruments Universal Analysis 2000, 4.7A. The non-isothermal thermo gravimetric curves were obtained using a simultaneous thermo balance module TG/ DTA, model Q600 (TA–Instruments).

4.1.2 Solubility studies:

Solubility studies of VH were conducted in various physiological buffers (pH 1.2HCl, pH 4.5 acetate buffer and pH 6.8 phosphate buffer). The sample solutions were prepared by adding

drug to 100 mL of physiological buffer and placed in an ultra-sonicator with a manual shaking for 30 min. The samples were filtered by a 0.45 µ nylon filter. Aliquots from transparent supernatant layer were analysed using HPLC at λ_{max} 216 nm [11].

4.1.3 Drug-Excipient compatibility studies:

The drug excipient compatibility studies were conducted for selection of excipients by using FT-IR and DSC studies. The combinations of drug with polymer or excipients physical mixture were recorded and analyzed [12].

4.1.3.1 Fourier Transform Infrared Spectroscopy:

FTIR study was conducted to explore any chemical interactions between drug and excipients. The physical properties of the drug, excipients and physical mixture were contrasted with those of plain drug. The FT-IR pure drug spectra and physical mixture of drug and excipients were analyzed using potassium bromide (KBr) disk approach. The process used in this preparation was by mixing of 2% w/w of sample with dry potassium bromide (KBr) IR powder; homogenous mixing by grinding in a mortar; eventually compacting under hydraulic press at 1000 or 12psi to produce a disk. The resulting disk was mounted in an appropriate holder and scanned using an FT-IR instrument Perkin-Elmer Model 1600 in the range 4000–500 cm-1 to obtain the characteristics of spectrum peaks and the resulting spectra was analyzed for functional groups & drug excipient compatibility.

4.1.3.2 Differential Scanning Calorimetry:

Differential Scanning Calorimetry (DSC) is a suitable thermal analysis technique for determining the purity, the polymorphic forms and the melting point of a sample. DSC was

used to examine any incompatibility between drug in combination with excipients. The DSC curves were obtained on a TA Instruments Calorimeter; model DSC Q20, using aluminium crucibles with about 2 ± 0.1 mg of samples under nitrogen atmosphere, at the flow of 50 mL min-1 with the heat ramp of 5°C/min. Data was analysed using the software TA Instruments Universal Analysis 2000, 4.7A. The non-isothermal thermo gravimetric curves were obtained using a simultaneous thermo balance module TG/ DTA, model Q600 (TA–Instruments), using alumina crucibles with about 8 ± 0.1 mg under a nitrogen atmosphere at 50 mL min-1.

4.2 Formulation analytical studies:

The formulation analytical studies were used to determine the quantitative estimation of drug in the finished product by HPLC

4.2.1 Assay method development: The mobile phase was prepared by a mixture of pH 3.0 triethylamine phosphate buffer and acetonitrile in the ratio of 60:40% v/v and filtered with vacuum through a 0.45 µm membrane filter and degassed in a sonicator for about 5 min [13].

4.2.2 Dissolution Method development: The mobile phase was prepared by a mixture of pH 3.0 triethylamine phosphate buffer, acetonitrile in the ratio of 60:40% v/v and filtered with vacuum through a 0.45 µm membrane filter and degassed in a sonicator for about 5 min.

The chromatographic parameters for assay and dissolution method development of VH minitablets are tabulated in (Table1).

Table 1. Chromatographic Parameters for assay and Dissolution method development of verapamil HCl minitablets

Chromatographic Parameters	Assay	Dissolution studies
Stationary phase	Inertsil ODS 3V 150 x 4.6 mm, 5 µm	Inertsil ODS 3V 150 x 4.6 mm, 5 µm
Mobile phase	60:40 v/v mixture of pH 3.0 triethylamine phosphate buffer, acetonitrile	60:40 v/v mixture of pH 3.0 triethylamine phosphate buffer, acetonitrile
Diluent	20:80 % v/v methanol: water	Dissolution Medium
Flow rate	1.3 mL/min	1.3 mL/min

Detection wavelength	216 nm	278 nm
Column temperature	30 ˚C	30 ˚C
Sample temperature	25 ˚C	25 ˚C
Injection volume and run time	20 μL, 15 min	10 μL, 6 min

4.2.3 Assay method validation parameters:

Specificity: Specificity was a measurement of the degree of interference from things such as other ingredients like excipients and drug standards, to check the interference blank, placebo and standard drug substance were prepared and injected.

System suitability: System suitability parameters were tested with six replicate injections of the diluted sample of working standards (50 μg/mL). The system suitability parameters were calculated using the internal feature of LC-solution software as per USP [14]. The parameters were retention time, peak area, and height, width at half peak height, tailing factor, efficiency, and height equivalent theoretical plates (HETP). System suitability was measured on the basis of precision (RSD). The precision, as measured by coefficient of variation was determined at each set's parameters and it should be less than 2% at the beginning of validation and at end of validation.

Precision:

Precision was determined by six replicate injections of sample solution. The precision (RSD) of the method was calculated.

Accuracy:

Accuracy was estimated by preparing and injecting low, medium, high concentrations of known amount of drug were studied. Accurately weighed 25 mg, 50 mg, 75 mg of VH with

excipients to get levels of 50%, 100%, and 150% w/w concentrations was transferred individually into 100 mL of volumetric flask and add 70ml of diluent, sonicated for 5 min. Further, 1ml from the stock solution was pipette out and diluted to 10 mL with diluent. The samples were injected into chromatographic system at λ_{max} 216 nm and recorded the chromatograms.

Linearity:

Standard curve for Verapamil HCl by using HPLC: Accurately weighed and transferred 50 mg of VH into a 100 mL of diluent and sonicated for 3 min, mixed well. An aliquot of 2 mL of this stock solution I was further diluted to 20 mL with diluent in order to obtain standard solution of 50 µg/mL. The various levels of solutions (5 to 62.5 µg/mL) were prepared by dissolving in diluent from standard solution of 50 µg/mL and the solutions were analyzed using an isocratic HPLC. The detection was carried out at λ_{max} 216 nm and the calibration curve for area vs. concentration (µg/mL) was plotted.

LOD and LOQ:

The LOD and LOQ of the developed methods were determined by analyzing progressively lower concentration of the standard solution using optimized chromatographic conditions. The minimum concentration of the standard solution, which gave signal to noise ratio of 3 and 10 were taken as the LOD and LOQ values respectively.

Robustness:

Capacity to remain unaffected by small but deliberate variations in method parameters. Comparison results under differing conditions with precision under normal conditions.

4.2   STABILITY STUDIES:

The final composition formula minitablets were filled in gelatin capsule size "0" and packed in HDPE bottle and placed at temperature 40±2°C up to 3 months. At the end of 3rd month capsules were subjected to assay and in-vitro release studies performed in pH 6.8PBS.

5.0 RESULTS AND DISCUSSION:

5.1 Identification Test (Melting point): The melting point determination is a very critical step in the drug formulation development. Since, this is the one of the steps in identifying the purity of the drug substance. The melting point of verapamil HCl was found around 144±2°C by capillary fusion method and also confirmed by DSC method (Fig 4). Altogether, confirms the purity of the drug substance, which is further strengthen by literature reports [15].

5.2 Solubility studies:

The solubility was observed as 3.07 mg/mL in water and 3.15 mg/mL, 3.18 mg/mL and 1.99 mg/mL in physiological buffer at pH 1.2, 4.5 and 6.8, respectively. The solubility in buffer is pH dependent and the solubility is increasing with lowering the pH (having lower solubility at pH 6.8, Fig 1) which could be due to its inherent alkaline pKa [16].
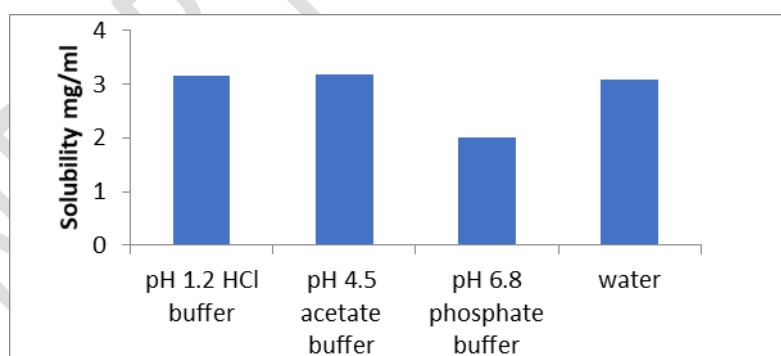

Fig.1. Solubility data of verapamil HCl in various physiological buffer solutions

The solubility of the drug substance drives the dissolution profile of the formulation [17].It is always very critical to identify an efficient solubility enhancer to minimize the pH dependent solubility [17]. Having pH dependent solubility of the drug substance would cause a significant change in dissolution profile especially when the formulation designed for

extended-release profile. Therefore, to minimize the pH dependent solubility of VH in drug product, pH modifier like fumaric acid was incorporated in formulation which assist in modifying the microenvironment around the drug substance and helps to improve the dissolution profile of drug product in pH 6.8PBS. It was supported by the *in vitro* dissolution profiles of VHMT formulation with (F1a) and without (F2) Fumaric acid with 10%w/w coating of EC shows in Fig.2
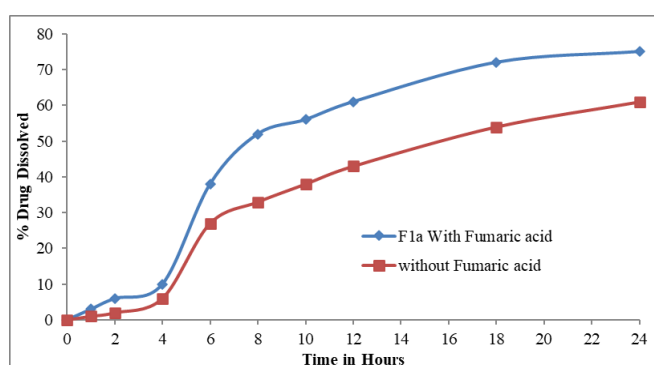

Fig.2. Comparative In vitro dissolution profiles of different concentration of fumaric acid in pH 6.8 phosphate buffer

5.3 Drug - excipients compatibility studies: The FT-IR spectrum of drug and the physical mixture of drug with polymer and excipients were shown in (Fig.3), reveals that the drug and excipients were compatible without any chemical interaction. The DSC thermogram of VH and VH + EC 100cps (controlled release polymer) shown in (Fig.4), reveals that no significant change in the melting point of VH was observed. From this it can be concluded that there exists no interaction between the drug and excipients used in the study.

Table 2. Compatibility studies of drug with excipients by infrared spectroscopy

S.NO	Functional group	Wave number cm^{-1} Verapamil hydrochloride	Wave number cm^{-1} Verapamil hydrochloride + All excipients
1.	N-H	3441	3412.40
2.	C-H	2959	2918.71, 2850.40
3.	C-O	1258.68	1269.06
4.	C=C	1519.04, 1596.52, 1461.94	1519.28

Table 3. Compatibility studies of drug with excipients by DSC

| S.NO	Drug and in combination with excipients	Endothermic peak at temp °C (melting point)
1.	Verapamil Hydrochloride	147.25°C
2.	Ethyl Cellulose	188.76°C
3.	PVP K30	82.52°C
4.	Microcrystalline Cellulose	99°C
5.	Magnesium Stearate	103.37°C
6.	Verapamil Hydrochloride + Ethyl Cellulose	146.8°C, 185.29°C
7	Verapamil Hydrochloride + Povidone K30	146.34°C, 90.43°C
8.	Verapamil Hydrochloride + Microcrystalline Cellulose	141.95°C, 94.83°C
9.	Verapamil Hydrochloride + Magnesium Stearate	142.49°C , 95.01°C
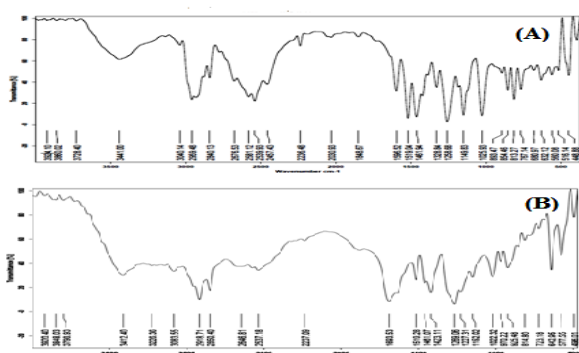
Fig. 3. FT-IR spectrum of (A) verapamil hydrochloride, B) Physical mixture of drug and excipients
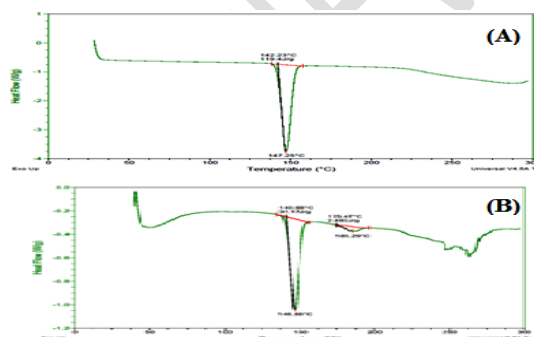
Fig.4. DSC Thermogram of: (A) Verapamil HCl, (B) verapamil HCl + ethyl cellulose

5.4 Formulation Analytical Method Validation Parameters (Assay):

Specificity: Specificity of the method was assessed by comparing the chromatograms obtained from capsule content and drug standards. The retention times of drug from standard solutions and from capsule content were identical and no coeluting peaks from the diluents were observed indicating specific method for quantitative estimation of drug in the commercial formulation.

System suitability: System suitability parameters were studied with six replicated standard solution of the drug and the calculated parameters are within the acceptance criteria. The tailing factor, the number of theoretical plates was in the acceptable limits (RSD less than 2%). The system suitability results are shows in table 4.

Table 4. System suitability results of Verapamil HCl minitablets

S.No	Parameters	VPH
1	Retention Time(min)	8.9
2	Resolution	---
3	Tailing factor	1.19
4	%RSD	0.82
5	Theoritical plates	9562

Precision: The samples of VH at 100% concentrations were estimated for percentage recovery. The average % recovery was found to be 99.87% and The % RSD was found to be 0.90% within the limits. The results are shown in (Table 5).

Table 5. Precision results of verapamil HCl obtained using HPLC

% Conc. (at specification level)	Area (mAU)	Amount added (mg)	% recovery	Average % recovery	%RSD
100%	1031278	50.31	99.67	99.87	0.90
	1042148	50.46	100.43		
	1050055	50.72	100.67		
	1032650	50.12	100.19		
	1012562	50.03	98.41		

Accuracy: The samples of VH at low, medium, high concentrations were estimated for percentage recovery. The average % recovery was found to be 99.34% within the limits. The results are shown in (Table 6).

Table 6. Accuracy results of verapamil HCl obtained using HPLC

% Conc. (at specification level)	Area (mAU)	Amount added (mg)	% recovery	% Mean recovery	Average % recovery
50%	509021	24.78	99.88	99.88	99.34
	539246	25.90	101.24		
	507759	24.70	99.96		
100%	1001278	49.10	99.16	99.16	
	1034148	50.21	100.15		
	1006475	49.18	99.51		
150%	1529057	75.10	99.00	99.00	
	1517716	74.51	99.05		
	1521140	75.12	99.11		

Linearity curve of verapamil HCl: Linearity curve of the VH observed from the concentrations of drug and response measured at λ_{max} 216 nm, using HPLC. A graph of conc. vs. area was plotted shown in (Fig.5). It was concluded that a perfect linearity between the concentration on x axis and area on y axis was observed when the concentration range was from the 5 to 75 µg/mL. The slope (k) and intercept (C) value was found to be 21384 and 8156. The correlation coefficient (r^2) value was found to be 0.999.
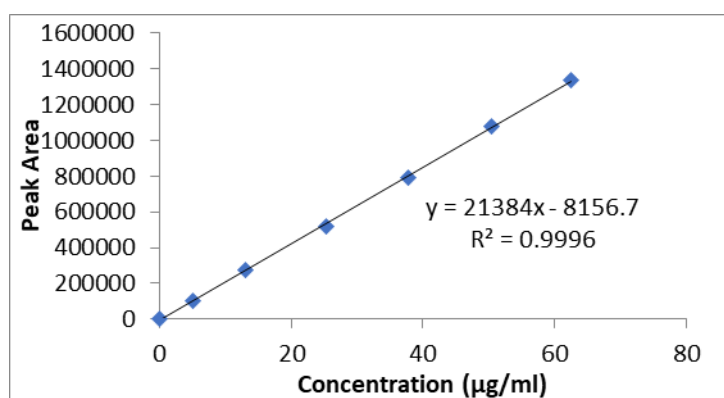
Fig.5. Linearity curve of verapamil hydrochloride

Chromatograms of blank, highest linearity 150% level conc., standard peak for dissolution, and optimized formulation's (F1) dissolution profile at 4 h, 12 h and 24 h were shown in (Fig.6)
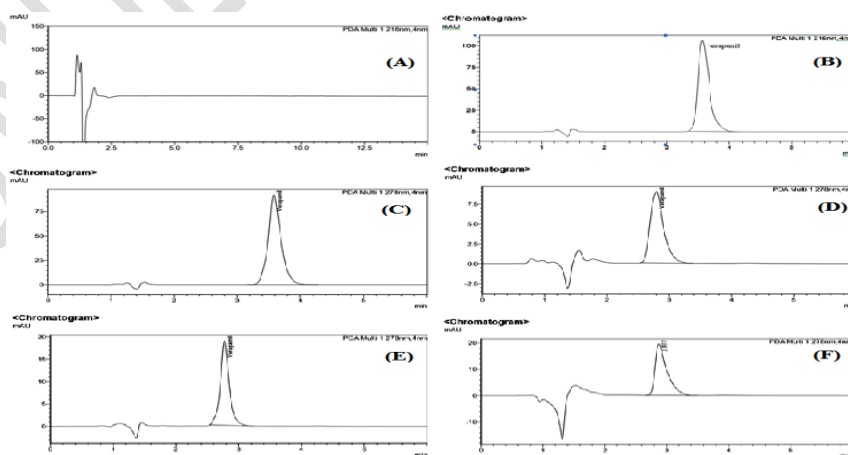

Fig.6. Chromatogram of: (A) Blank, (B) Highest linearity 150% level conc., (C) Standard peak for dissolution, and Optimized formulation (F1a) dissolution profile at (D) 4 h, (E) 12 h and (F) 24 h

LOD and LOQ: The LOD and LOQ of the developed methods were determined by analysing progressively lower concentrations of the standard solutions using optimized chromatographic conditions. The minimum concentration of the standard solution, which gave signal to noise ratio of 3 and 10 were taken as the LOD and LOQ values respectively. LOD and LOQ values of verapamil was found LOQ 0.06 µg/mL and LOD 0.018µg/mL.

Robustness: The robustness study we had compared the results between normal operating conditions and by deliberately changing certain parameters like flow rate and mobile phase buffer pH. The result obtained shows that by changing deliberately some internal parameters of the method does not influence the results obtained (Table 7).

S.No	Condition	Variation	Average area	% RSD
1	Mobile phase Phosphate buffer(pH3.0):Acetonitrile(60:40)	Phosphate buffer(pH3.0):Acetonitrile(55:45)	1046321	1.12
		Phosphate buffer(pH3.0):Acetonitrile(60:40)	1078898	0.98
		Phosphate buffer(pH3.0):Acetonitrile(65:35)	1095687	1.06
2	Flow rate 1.3ml/min	Minus Flow rate 1.2ml/min	1125693	1.32
		Flow rate 1.3ml/min	1078898	0.98
		Plus Flow rate 1.4ml/min	1057630	1.26

Table 7. Robustness results of verapamil HCl obtained using HPLC

5.4 Stability studies:

The stability studies were performed on formulation of verapamil mini tablets at temperature 40 ± 2^0C up to 3 months for analyzed assay and in-vitro release performance of the drug product. All the results were showed within the acceptance limits and Invitro dissolution profile (Fig 7) similarity factor F2 more than 50 when compared against the initial results.

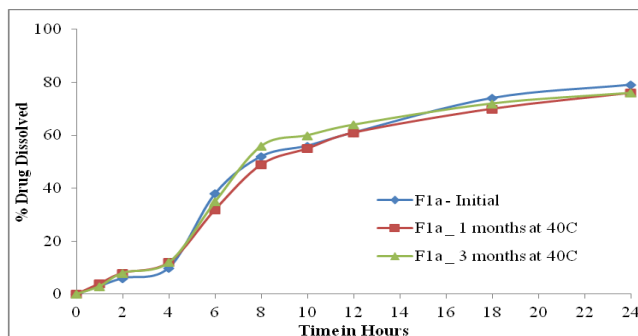| Table 8 : Assay value of stability sample @40/75%RH	
Condition	Assay Value
Initial	101%
40°C/75% RH – 1 Months	99%
40°C/75% RH – 3 Months	99%

Fig.7.In-vitro dissolution profile of stability studies of Optimized Formulation (F1a) at 40±20C of 1and 3 month in pH 6.8 phosphate buffer

Conclusion: The pulsed release formulation of VH was developed and the formulation met the desired release characteristics. The various pre-formulation analytical techniques have been used during early development viz. melting point, solubility and excipient compatibility. The pre-formulation analytical techniques like capillary fusion method and DSC method helped to identify the purity of drugs substance to work with, which is the primary most important step prior to the start of formulation development process. Later, the solubility of the drug substance was evaluated at various physiological buffers at various pH and found the drug substance shown a pH dependent solubility, which would pose many challenges in later stage of the formulation development like poor dissolution characteristics in alkali conditions. Therefore, the early identification of this shortcoming helped the formulation scientist to come-up with fumaric acid as an additive to maintain the microenvironment of formulation by which the pH dependent solubility problem was overcome at early stages. Later, the compatibility studies were performed and found to be acceptable with minimal or no interaction. Further, the developed formulation drug release characteristics were verified by formulation analytical techniques viz. percent assays and dissolution profiles. Overall, all these analytical techniques helped the formulation scientist to identify the major defects (pH

dependent solubility) at early stages and overcome those at early which minimizes the significant associate cost and resources.

References:

1. Boyette LC, Manna B. Physiology, Myocardial Oxygen Demand. [Updated 2021 Jul 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499897/

2. Ramon C. Hermida, Diana E. Ayala, Carlos Calvo, Francesco Portaluppi, Michael H. Smolensky. Chronotherapy of hypertension: Administration-time-dependent effects of treatment on the circadian pattern of blood pressure. *Adv Drug Del Rev*. 2007; 59: 923-939.

3. Hermida RC, Smolensky MH. Chronotherapy of hypertension. Curr Opin Nephrol Hypertens. 2004 Sep;13(5):501-5. doi: 10.1097/00041552-200409000-00004. PMID: 15300155

4. Burnier M. Long-term compliance with antihypertensive therapy: another facet of chronotherapeutics in hypertension. Blood Press Monit. 2000;5 Suppl 1:S31-4. PMID: 10904240.

5. D. K. Singh, Sushilkumar S. Poddar. Development of biphasic pulsatile release mini-tablets system of poorly water soluble carvedilol phosphate. *Int J Pharm Sci Res*. 2015; 6(5):919-926.

6. Md. Medhi Hasan, Harun AR Rashid Pharmaceutical Preformulation for Product Development & Analytical Techniques use in new Dosage form. International Journal of Pharmacy & Therapeutics. January 2017; 8(1):16-32.

7. P.Tharun sankeerth, N.Bhavana, P.V.suresh, N.RamaRao Preformulation analytical Techniques during Drug Development. International Journal of pharmacy & Pharmaceutical Research; March 2017 Vol.: 8, Issue:4: 107-125.

8. Haimhoffer, Á.; Dossi, E.; Béresová, M.; Bácskay, I.; Váradi, J.; Afsar, A.; Rusznyák, Á.; Vasvári, G.; Fenyvesi, F. Preformulation Studies and Bioavailability Enhancement of Curcumin with a 'Two in One' PEG-β-Cyclodextrin Polymer. *Pharmaceutics* **2021**, *13*, 1710. doi.org/10.3390/pharmaceutics13101710.

9. Nadendla, R. R., Satynarayana, J. and Burri, J. K. (2022) "Rivaroxaban: Compatibilty with Pharmaceutical Excipients using DSC and FTIR Spectrophotometry", Journal of Pharmaceutical Research International, 34(12A), pp. 43-50. doi: 10.9734/jpri/2022/v34i12A35554.

10. Nadendla, R. R., Pinnamaneni, P., Morla, S. P. and Abhinandana, P. (2021) "Physico-Chemical Characterization of Paliperidone Palmitate and Compatibility Studies with its Pharmaceutical Excipients", *Journal of Pharmaceutical Research International*, 33(5), pp. 85-91. doi: 10.9734/jpri/2021/v33i531183.

11. Nitin D. Jagadale, Dipak D. Gadade, Ashish A Heda, P. K. Puranik. Formulation Development and Evaluation of Sustained Release Pellets of Verapamil HCl. *Int J Pharm Res Dev*. 2010; 1(11): 1-7.

12. Li CJ, Zhu JB, Yu WY. Preparation of verapamil hydrochloride core-in-cup tablets with double-pulsatile and multi-phasic release. *Yao Xue Xue Bao*. 2008; 43(6): 652-6.

13. Kondeti Ranjith Reddy, Grace Rathnam, I. Kiran, Shalem Raju, Kranti Sri Mulpuri. Formulation Development and Evaluation of Sustained Release Matrix Tablets of Verapamil Hydrochloride. *Int J Pharma Sci Res*. 2014; 5(5): 2066-73.

14. United states pharmacopoeia, "United states pharmacopeial convention," Tech. Rep.USP 27,NF22, United states pharmacopoeia, Rockville,Md,USA,2004.

15. Verapamil hydrochloride, 152-11-4-Chemical Book; https://www.chemicalbook .comP.

16. Verapamil hydrochloride , Drug Bank Online; https://go.drugbank.com>salts>DBSALT00534.

17. Lin Z, Zhou D, Hoag S, Qiu Y. Influence of Drug Properties and Formulation on In Vitro Drug Release and Biowaiver Regulation of Oral Extended Release Dosage Forms. *AAPS J*. 2016;18(2):333-345.