Original Research Article

Analytical tools used for characterization and development of Minitablets: A Verapamil Hydrochloride Case Study

1. Abstract:

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 Fourier transform infrared(FTIR) and Differential scanning calorimetry （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. A part of physicochemical properties, the solubility data of verapamil hydrochloride(VH) 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 of VH. 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 like a stability indicating HPLC assay procedure has been developed and validated for VHMT life cycle (initial and stability samples). The analytical data of stability samples of VHMT showed stable upto 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. In conclusion, 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, pre-formulation, 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 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]. 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, acid dissociation constant (Pka), particle size, crystalline nature, excipient compatibility (by Fourier Transform Infrared Spectroscopy and Differential Scanning Calorimetry). 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. High performance liquid chromatography (HPLC) grade dichloromethane were purchased from Sigma Chemical Industries, Hyderabad.

4. Experimental procedure:

4.1 Pre-formulation 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 these stages 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, CF method and DSC method.

Capillary fusion (CF) 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 ^{o} C/min.

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 Fourier transform infrared and Differential scanning calorimetry 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):

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 were analyzed for functional groups and 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 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), 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 verapamil hydrochloride minitablets are tabulated in (Table1).

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

Chromatographic Parameters	**Assay**	**Dissolution studies**
Stationary phase	Inertsil ODS 3V 150x4.6 mm, 5µm	Inertsil ODS 3V 150x4.6 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 is 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 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 hydrochloride 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.

Limit of Detection (LOD) and Limit of Quantitation (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 6.8(phosphate buffer solution) PBS.

5.0 RESULTS:

5.1 Identification Test (Melting point):

The melting point of verapamil hydrochloride was found to be 144 ± 2°C by capillary fusion method and the DSC curve exhibited and endothermic peak at 144 (Fig 4).

5.2 Solubility studies:

The solubilities were observed as 3.07 mg/mL in water, 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 (Fig.1).

5.3 Drug - excipients compatibility studies:

The FTIR spectrum of drug and the physical mixture of drug with all excipients were depicted in (Fig.3 A,B), FTIR spectra of pure drug showed characteristic absorption bands located at 3441 cm^{-1} for secondary amine (N-H) stretching, 1596.52, 1519.04 and1461.94 cm-1 corresponding to C=C stretching from the alkene group, 1258.68 and 1149.83 cm^{-1} corresponding to C-O stretching from the ester group, whereas -CH3 appeared at wave number of 2959.48 cm-1 (Fig 3 A,B). Similar spectral peaks observed in all VH - physical mixture with all excipients (Table 2). The DSC thermogram exhibits an endothermic peak at 147.35°C for VH, corresponding to melting temperature of reference and above similar endothermic peak was exhibits for VH + EC 100cps (controlled release polymer) (Fig.4 A,B) and other physical mixture combinations showed similar endothermic peak (Table 3).

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 co-eluting 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 system suitability results are shown in (Table 4).

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).

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).

Linearity curve of verapamil hydrochloride:

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).

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)

Limit of detection (LOD) and Limit of quantification (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 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).

5.5 Stability studies:

The stability studies were performed on formulation of verapamil hydrochloride minitablets at temperature 40 $\pm 2^0$C up to 3 months for analyzed assay and in-vitro release performance of the drug product. All the results were showed in Fig 7 and Table 8.

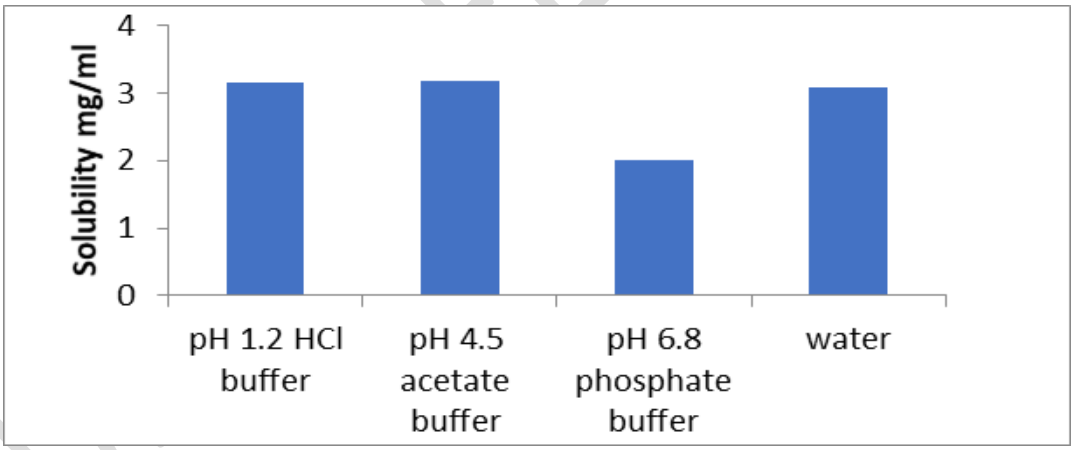

Fig.1. Solubility data of verapamil hydrochloride in various physiological buffer solutions

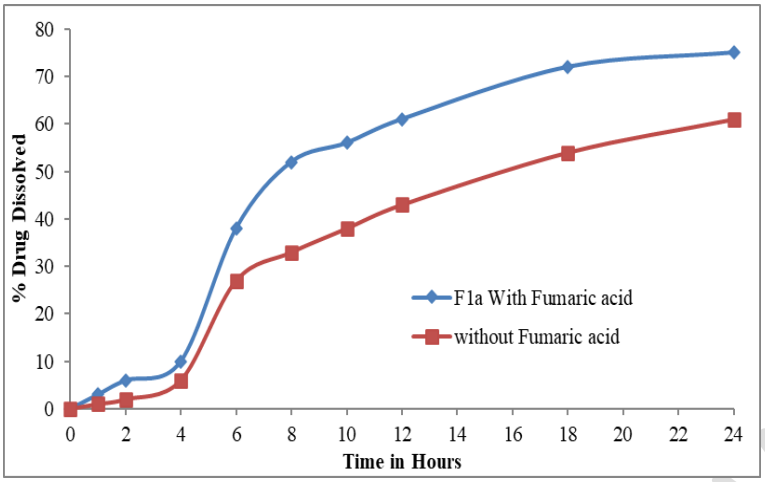

Fig.2. Comparative In vitro dissolution profiles of different concentration of fumaric acid in pH 6.8 phosphate buffer

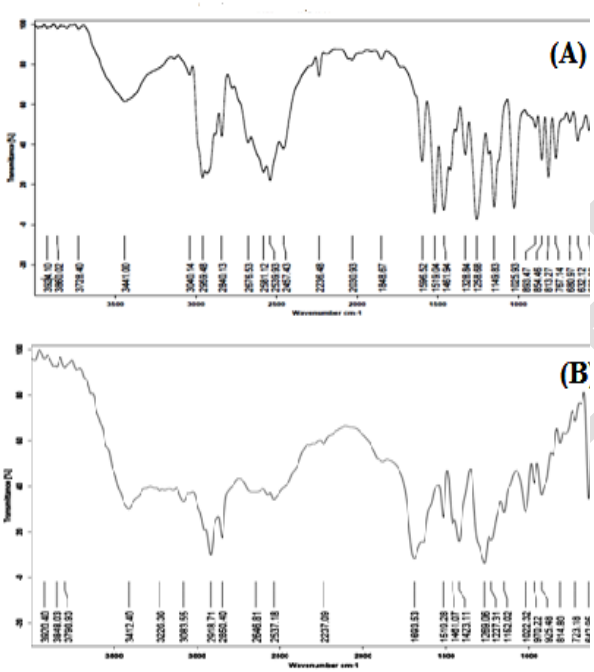

Fig. 3. FT-IR spectrum of (A) verapamil hydrochloride, B) Physical mixture of drug and excipients

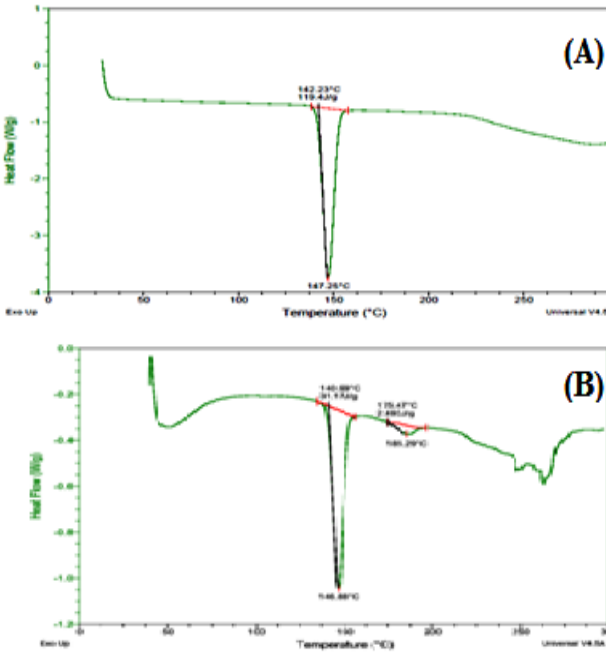

Fig.4. DSC Thermogram of: (A) Verapamil HCl, (B) verapamil HCl + ethyl cellulose

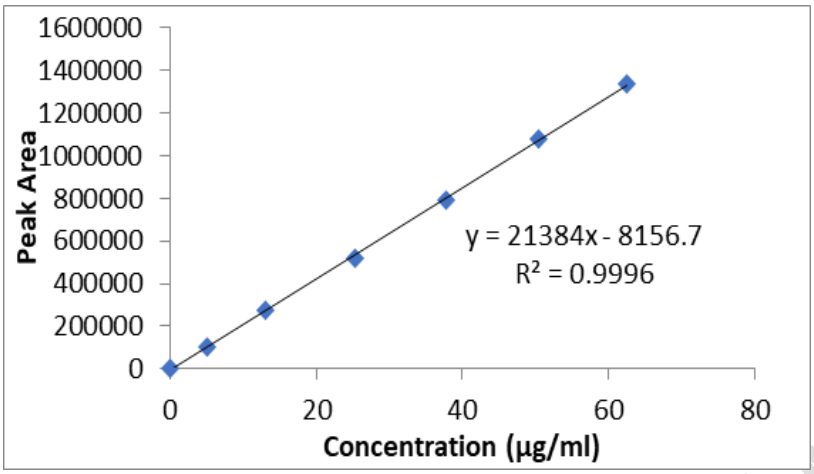

Fig.5. Linearity curve of verapamil hydrochloride

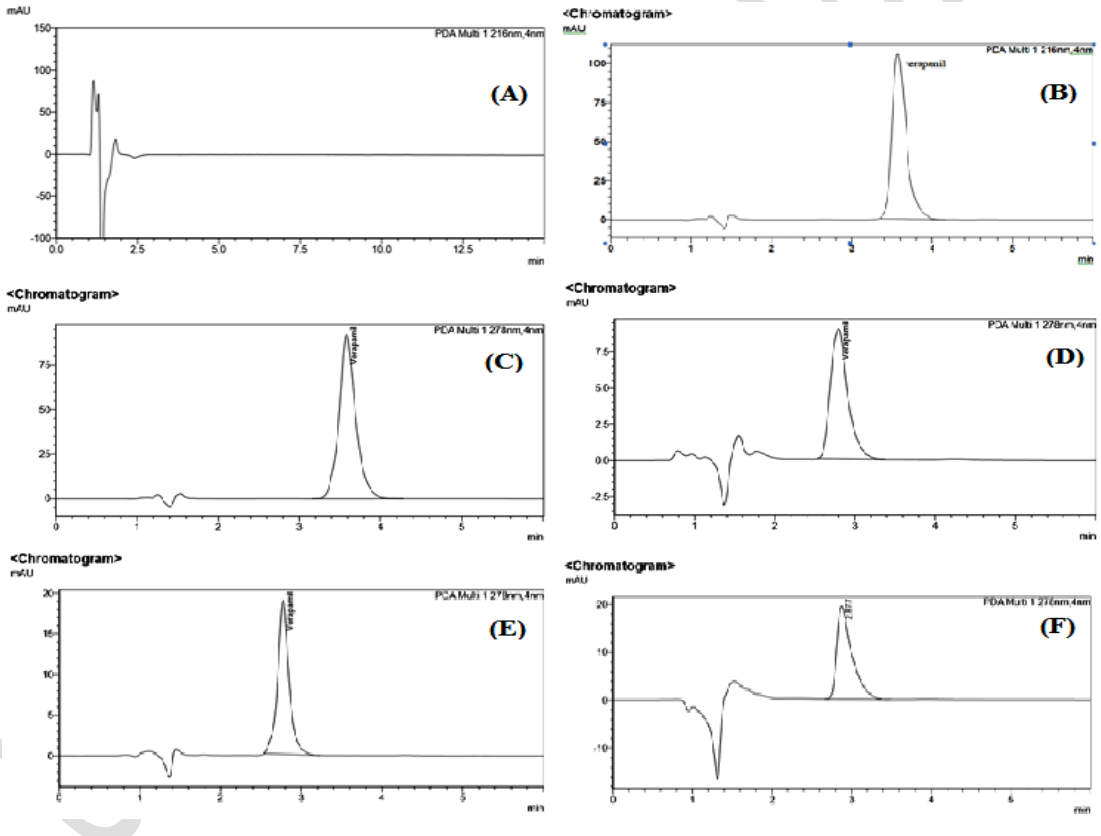

Fig.6. Chromatogram of: (A) Blank, (B) Highest linearity 150% level conc., (C) Standard peak for dissolution, and Optimized formulation (F1a) dissolution profile at (D) 4 h, (E) 12 h and (F) 24 h

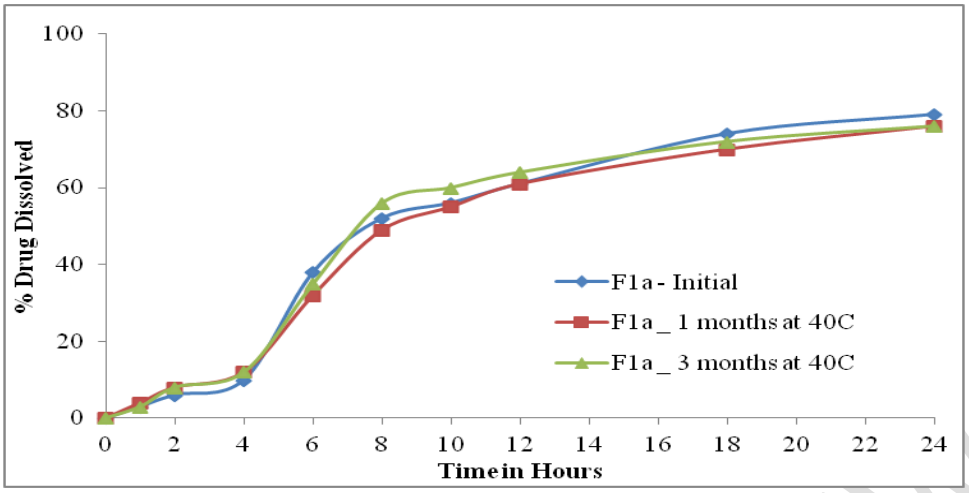

Fig.7.In-vitro dissolution profile of stability studies of Optimized Formulation (F1a) at 40±20C of 1and 3 month in pH 6.8 phosphate buffer

Table 2. Compatibility studies of verapamil hydrochloride 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

Note: N-H secondary amine, C-H hydrocarbon, C-O ester and C=C alkene

Table 3. Compatibility studies of verapamil hydrochloride 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

Table 4. System suitability results of verapamil hydrochloride minitablets

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

Table 5. Precision results of verapamil hydrochloride 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		

Table 6. Accuracy results of verapamil hydrochloride 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		

Table 7. Robustness results of verapamil hydrochloride obtained using HPLC

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 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%

6. Discussion:

The pre-formulation analytical study data like, the melting point of verapamil hydrochloride was found to be 144 ± 2°C by capillary fusion method and also confirmed by DSC method (Fig 4). It confirms the purity of the drug substance, which is future strengthening by literature reports [15]. The solubility data of verapamil hydrochloride at various physiological buffers Fig 1, illuminates a pH dependent solubility (low soluble in pH6.8 Phosphate buffer), which could be due to its inherent alkaline pKa [16]. The early identification of this shortcoming helped 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 of development. The solubility of the drug substance drives the dissolution profile of the formulation [17]. 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. The compatibility study results Table3 showed the endothermic peak of drug substance (Verapamil HCl) and various drug excipient physical mixture. The DSC thermogram confirm that there is no physicochemical interaction between sharp endothermic peak at 147.25°C (Fig.4). Due to low level of interaction in each component of the physical mixture minute changes in the melting endothermic peak of verapamil HCl were observed. The FTIR spectrum of VH showed characteristic absorption bands 3441 cm-1, 2959 cm^{-1}, 1652.57 cm^{-1}, 1519.04 cm^{-1}, 1596.52cm^{-1} and 1258.68cm^{-1} given in Table 2. The FTIR spectral peaks in drug substance and physical mixture were mapped. Thus the absence of any new peaks or absence of shifts in the FTIR peaks of the

physical mixture compared with VH indicated the lack of interactions (Fig.3).The Drug excipient compatibility studies relives that no chemical interaction between VH and selected pharmaceutical excipients. The formulation analytical method like assay was validated for reproducibility and repeatability. A linearity range of 5 to 75µg/ml with correlation coefficient 0.9996% was established for verapamil hydrochloride (Fig.5). The system suitability parameters like retention time, tailing factor and theoretical plates were in the acceptable limits RSD <2% (Table.4). The precision was carried in terms of repeatability. The % RSD 0.90 reveals that the proposed method is precise (Table.5). The % recovery was found to be 99.34% within the limits (Table.6). The results of robustness in the present method showed no significant changes (Table.7). The results of specificity of VH indicated that no interference with VH and excipients was observed with the developed method (Fig.6). The validated formulation analytical method was used for quantification (Assay) and invitro dissolution study of VHMT stability samples, all the results were showed within the acceptance limits (Table.8). The similarity factor F2 more than 50 for invitro dissolution profile when compared against the initial results (Fig 7).

7. 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, helped to identify the deficiencies and overcome those at early stages. Further, the developed formulation drug release characteristics were verified by formulation analytical techniques viz. assay and dissolution profiles. In conclusion, all these analytical techniques helped the formulation scientist to identify the major defects (like pH dependent solubility) at early stages and overcome those at early which minimizes the significant associate cost and resources.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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