Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN Print 2231 – 3648
Online 2231 – 3656

Application of cocrystal technique in the enhancement of solubility of spironolactone in drug release by UV spectrophotometric method.

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ABSTRACT

The main aim of the thesis is to enhance the solubility of "Spiranolactone" by formulation of co-crystals and to validate the spiranolactone co-crystals by UV-spectrophotometric method. The design of new multicomponent crystal phases of Spiranolactone with desired physicochemical properties by applying crystal engineering technique. Capability to design new multicomponent crystal structures will depend mostly on supramolecular chemistry. The development of new supramolecular complexes, co-crystal and polymorphs of drugs by crystal engineering is becoming progressively more important as an alternative to salt formation, mainly for neutral or weakly ionisable compounds. Validation of the developed UV method was done as per the ICH guidelines Q2(R1). The validation parameters such as linearity, LOD, LOQ, precision and accuracy were evaluated. Linearity and range of the methods were analysed by preparing calibration curves using different concentrations range of standard Spiranolactone-Succinic acid(10-50µg/ml) at 245nm. The calibration curve was plotted using peak area and concentration of the standard solutions. It was concluded that the perfect linearity was observed between the concentration and peak area and the concentration range from 10-50µg/ml. The results revealed that linear regression equation for spiranolactone-succinic acid was Y=0.006x and co-relation co-efficient (R^2) value 0.983. Precision studies were carried out and the mean, standard deviation (SD) were calculated and found to be within the limit. The results revealed that percentage co-efficient of variation(%RSD) was found to be >2%. Accuracy is reported as % recovery of the analysed concentration. The results indicate that the recovery of spiranolactone-saccharin was consistent at all levels and the percentage recovery for spiranolactone-saccharin was in between 40-110%.

Keywords: supramolecular complexes, cocrystal technique, solubility enhancement, Spiranolactone

INTRODUCTION

A co-crystal is a crystalline structure composed of at least two components, where the components may be atoms, ions or molecule. A more inclusive definition is that co-crystals "consist of two or more components that form a unique crystalline structure having unique properties".[1] In the framework of co-crystals, supramolecular synthesis is relatively low-risk strategy, as the approach employs theories of molecular recognition and self assembly rather than creating covalent bonds. A complete understanding of the supramolecular chemistry of the functionalities present in given molecule is the first step in designing a co-crystal since it facilitates choice of molecules that contain the appropriate complimentary functionalities. Here in, these complimentary molecules will be referred to as co-crystal formers. Co-crystals may be characterized in a wide variety of ways (X-ray diffraction, FTIR etc.,) The components interact via non-covalent interactions such as hydrogen bonding, ionic interactions, Vander Waals interactions and π-interactions. The intermolecular interactions and resulting crystal structures can generate physical and chemical properties that differ from the properties of the individual components [2]. Such properties include melting point, solubility, chemical stability, and mechanical properties. Some co-crystals have been observed to exist as polymorphs,

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which may display different physical properties depending on the form of the crystalline structure having unique properties [2]. The main aim of the thesis is to enhance the solubility of "Spiranolactone" by formulation of co-crystals and to validate the spiranolactone co-crystals by UV-spectrophotometric method.

Production of co-crystals

Crystallisation with molar excess
Crystallizing with a molar excess of one co-crystal former may produce a co-crystal by a decrease in solubility of that one component.

Crystallisation in a slurry
In crystallisation process, solvent plays an important role. Changing the solvent will change the intermolecular interactions and possibly lead to formation of co-crystals.

Cooling
Cooling the mixture of co-crystal formers and drug often leads to formation of co-crystals.

Seeding
A small piece of crystal called seed crystal can be grown into a large crystal by dipping it in a supersaturated solution of molten material and then cooled[2].

Grinding
Grinding by liquid-assisted method is employed to produce co-crystal e.g., using a mortar and pestle, ball mill or vibratory mill. In liquid-assisted grinding, a small amount of solvent is added to the grinding mixture. This method was developed in order to increase the rate of co-crystal formation[4].

Super critical fluids
Supercritical fluids (SCFs) serve as a medium for growing co-crystals. Crystal growth is achieved due to unique properties of SCFs by using different supercritical fluid properties[5][6].

Slow evaporation
Co-crystals are obtained by slow evaporation of solution containing two components. This method is applicable for molecules having complimentary hydrogen bonding properties, in which co-crystallization is likely to be thermodynamically favoured [3].

Analytical method development
Analytical chemistry is the branch of Science that uses advance technologies in determining the composition by analytical technique. Both qualitative as well as quantitative results can be performed. Analytical instruments play a major role in the process to achieve high quality and reliable analytical data. Quality assurance of equipment must be concerned.

Analytical method could be spectral, chromatographic, electrochemical, hyphenated or miscellaneous. Analytical method development is the process of selecting an accurate assay procedure to determine the composition of a formulation. It is the process of proving that an analytical method is acceptable for use in laboratory to measure the concentration of subsequent samples. Analytical methods should be used within GMP and GLP environments and must be developed using the protocols and acceptance criteria set out in the ICH guidelines Q2(R1).

VALIDATION PARAMETERS[8]

Linearity and Range
The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample.

Accuracy
Accuracy refers to the closeness of a measured value to the standard or known value (or) Closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

$$\text{Percentage recovery} = (C-B)/A*100$$

Where,

C= mixed solution of spiranolactone and co-crystals
B= solution of spiranolactone
A= solution of co-crystal

Precision
The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision may be considered at three levels

- System precision
- Method repeatability
- Intermediate precision
- Reproducibility

$$\text{Percentage relative standard deviation} = \text{Standard deviation/mean}*100$$

Limit on detection and limit on quantitation

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD=3.3*N/S

Where,

N=standard deviation of peak areas of drug
S=slope.

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

LOQ=10*N/S

Where,

N=standard deviation of peak areas of drug
S=slope.

Robustness/Ruggedness

The robustness/ruggedness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Specificity

The ability of an analytical method to distinguish the analyte from other chemicals in the sample is known as specificity.

METHODOLOGY

Formulation Of Multicomponent Crystal Forms Of Spiranolactone

Crystallization can happen from the melt or from the solution, or from the vapor phase. Crystallization can produced from the solution by lowering the temperature, removing the solvent from solution (this is also called evaporation), by an antisovent addition method (drowning out), by reactive crystallization (precipitation), or by altering the solution pH (isoelectric precipitation method).In this study solution crystallization (slow evaporation) method has followed to prepare following crystal forms of spiranolactone.

Characterization Of Multicomponent Crystal[10] Forms Of Spiranolactone

Spiranolactone–Saccharin (1:1), Spiranolactone-Succinic acid (1:1), Spiranolactone-L(+) Tartaric acid (1:1) co-crystals were characterized by research microscope, FTIR, UV studies with previously mentioned methods.

Research microscope

Individual co-crystals of spiranolactone-saccharin, spiranolactone-(L+) Tartaric acid and spiranolactone-succinic acid were collected and analyzed by research microscope in specific magnifications (10X, 40X)

FTIR

Individual co-crystals were collected and characterized by Agilent cary60 FTIR in the range of 400-4000cm^{-1}.

Aqueous Solubility

For the determination of drug content, prepared co-crystals (10mg), were dissolved in 100ml of water and the solution was analyzed spectrophotometrically at 245nm (λmax) for drug content, after sufficient dilution with distilled water.[7]

UV METHOD DEVELOPMENT

Selection of solvent

Methanol is selected as a solvent for drug. Water is selected as a solvent for the synthesized co-crystals.

Selection of wavelength

For many samples, good analytical results will be obtained only by careful selection of the wavelength used for detection. This choice requires a knowledge of the UV spectra of individual samples component. UV spectra can be measured prior to other methods.Standard solution of drug or co-crystals were prepared and scanned in UV spectrophotometer between 200-400nm using water as a blank.

VALIDATION PARAMETERS

Validation for the method was carried out as per ICH Q2 (R1) guidelines. The validation parameters such as linearity, accuracy, precision, LOD, LOQ are carried out as per the guidelines.

Linearity

Suitable aliquots of the stock solution of spiranolactone co-crystals were taken in 25ml volumetric flasks and the volume was then made up to the mark with distilled water to prepare a series of standard solutions in the concentration range of 10-50µg/ml. The calibration curve was plotted against concentration vs peak area and the intercept, slope values were noted.

Precision

The closeness of agreement between a series of measurements. Multiple sampling of homogenous samples under prescribed condition. The precision method was evaluated by inter day and intraday variation studies. In intraday studies, working solution of standard and sample were analyzed thrice in a day and %RSD was calculated. In

inter day variation studies, working solution of standard and sample were analyzed on three consecutive days and %RSD was calculated.

%RSD = standard deviation/mean*100

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3*σ/slope

Where,

σ = the standard deviation of the response
S = the slope of the calibration curve

Limit of quantitation

The limit of quantitation of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities or degradation product.

LOQ = 10*σ/slope

Where,

σ = the standard deviation of the response
S = the slope of the calibration curve

Accuracy

Accuracy normally refers to the difference between the mean of the set of results and the true or correct value for the quantity measured. Accuracy of the developed method was determined based on the recovery studies. Recovery studies were carried out by using known concentration of standard solution to the sample. The results of accuracy were noted for 50%, 100%, 150% and %recovery was calculated.

The %Recovery was calculated based on the following formula

%Recovery = (C-B) /A*100

Where,

C= absorbance of mixture of co-crystal and drug.
B= absorbance of standard drug solution.
A= absorbance of synthesized co-crystals solution.

RESULTS AND DISCUSSION

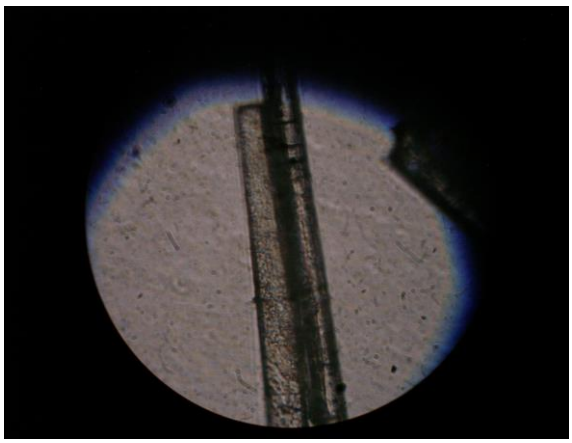

Fig 1: Spiranolactone-saccharin co-crystal at 10X magnification

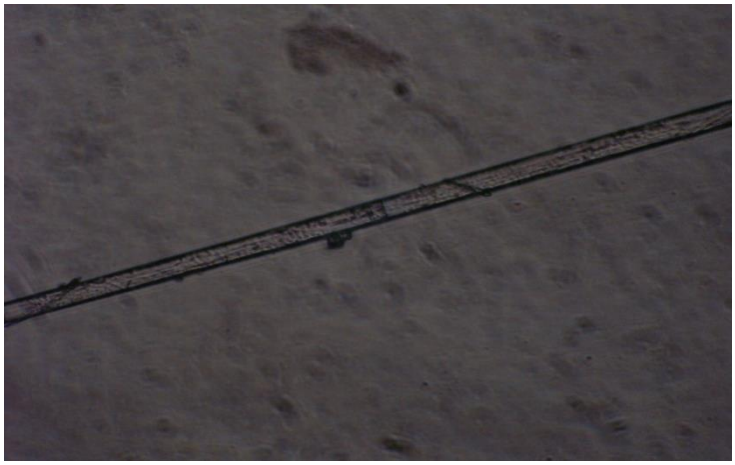

Fig 2: Spiranolactone-Tartaric acid co-crystal at 10X magnification

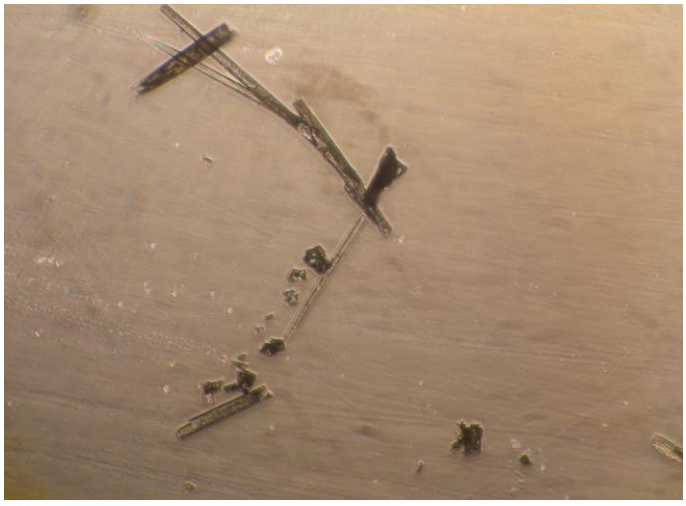

Fig 3: Spiranolactone-Succinic acid co-crystal at 45X magnification

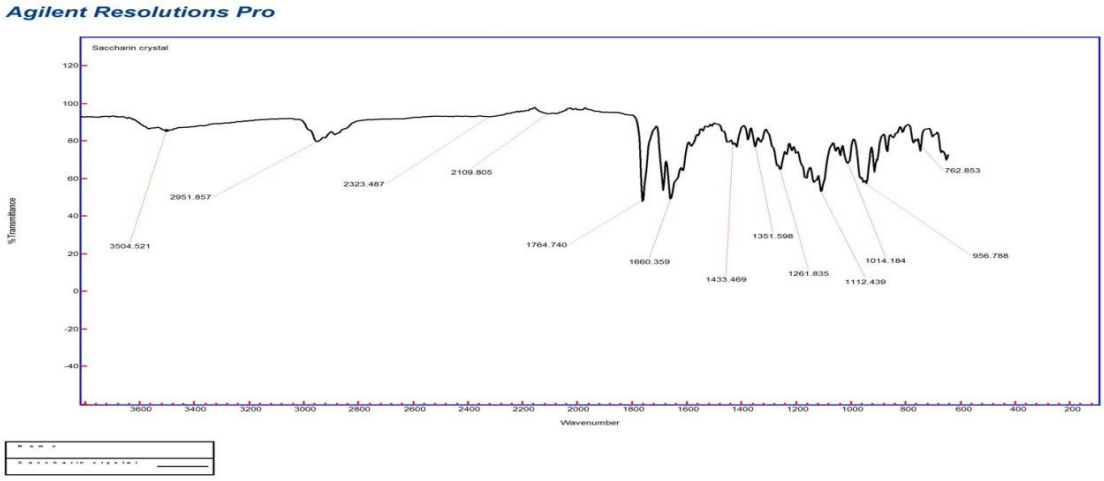

Fig 4: IR spectra of Spiranolactone-Saccharin co-crystal

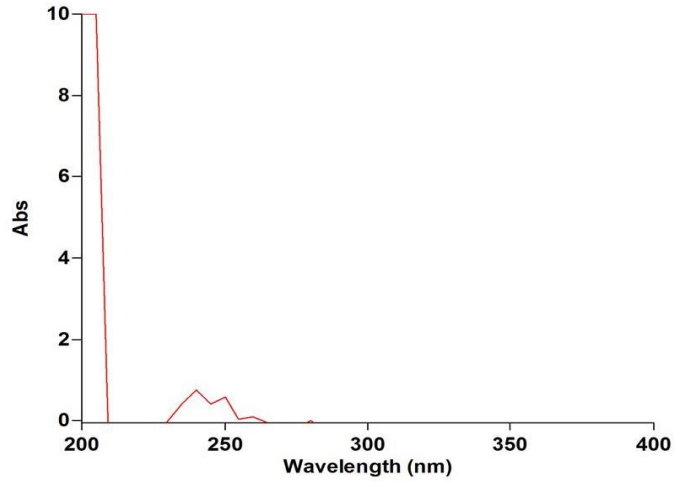

Fig 5: UV spectra of Spiranolactone-Saccharin co-crystal

Parameter	Observed value
λmax	240nm

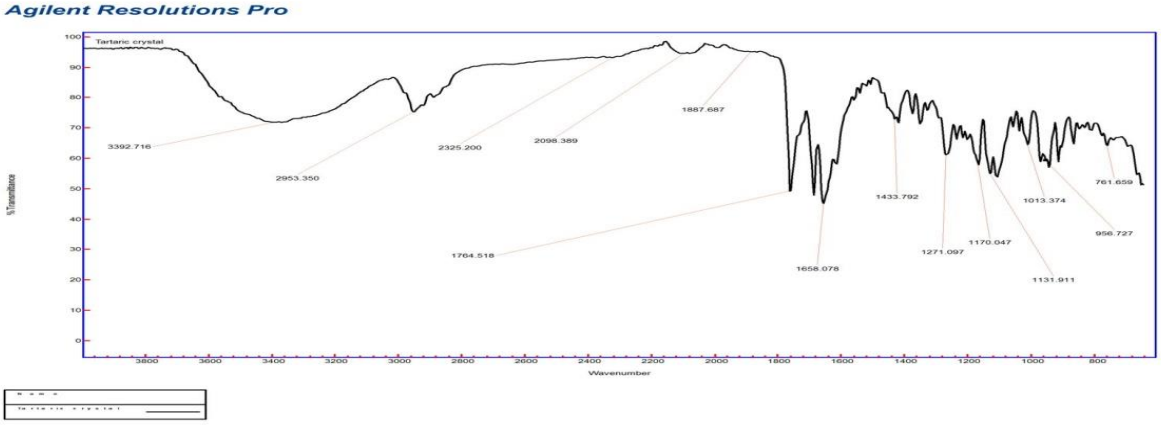

Fig 6: IR spectra of Spiranolactone-Tartaric acid co-crystal

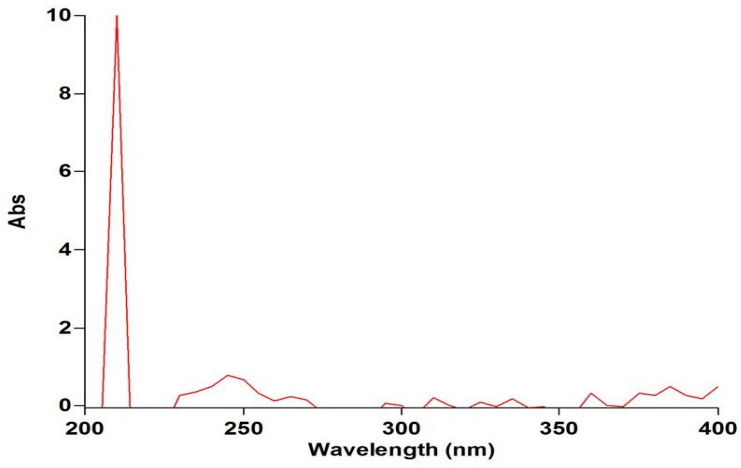

Fig 7: UV spectra of Spiranolactone-Tartaric acid co-crystal

Parameter	Observed value
λmax	245nm

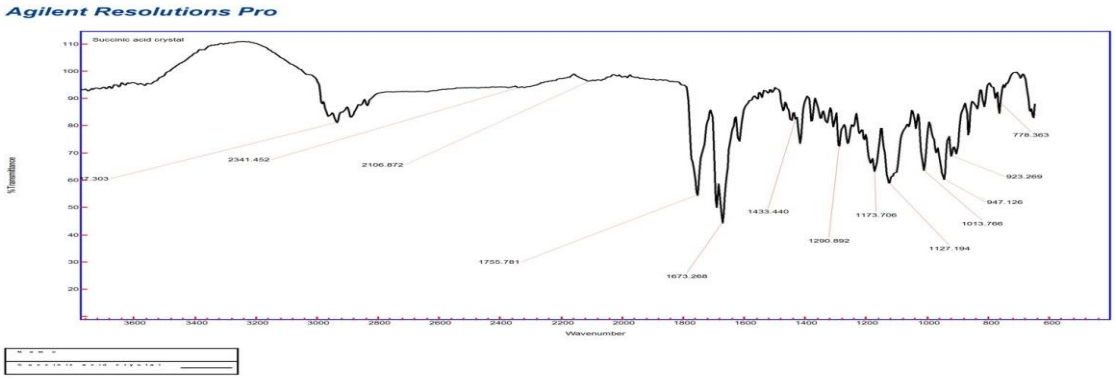

Fig 8: IR spectra of Spiranolactone-Succinic acid co-crystal

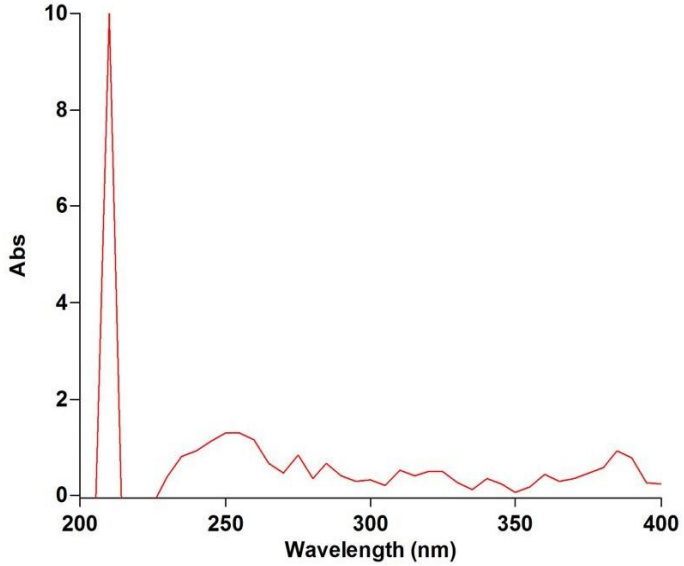

Fig 9: UV spectra of Spiranolactone- Succinic acid co-crystal

Parameter	Observed value
λmax	255nm

Aqueous solubility study

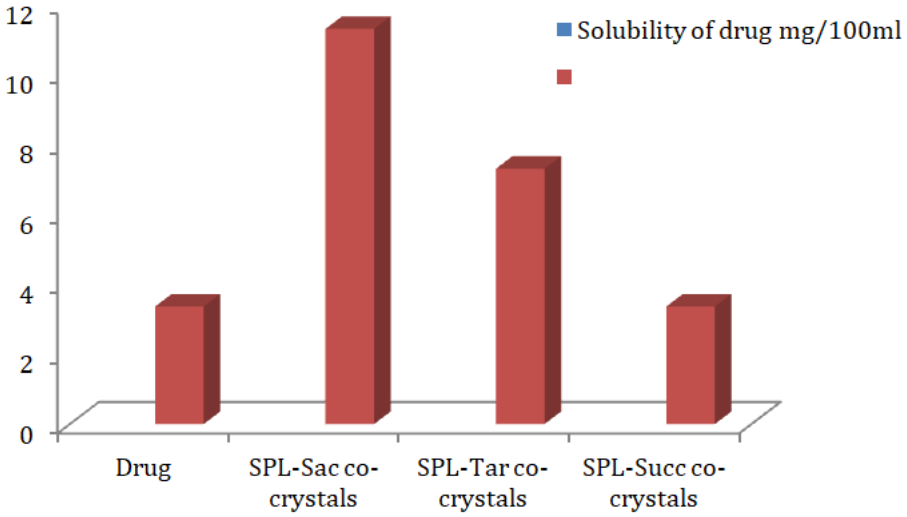

Fig 10: Solubility of drug and its co-crystals

Table 1: Linearity

	Linearity		
Co-crystals	**Spiranolactone-Saccharin**	**Spiranolactone-Tartaric Acid**	**Spiranolactone-Succinic Acid**
y	0.008x	0.008x	0.006x
R^2	0.970	0.999	0.983

Table 2: Precision studies

Co-crystals	Concentration(µg/ml)	Mean	SD	%RSD
Saccharin	10	0.1114	$4.5738x10^{-3}$	4.165
	30	0.2949	0.01395	4.730
	50	0.4846	0.02502	5.163
Tartaric acid	10	0.0434	0.08637	199.009
	30	0.2693	0.01265	4.697
	50	0.456	0.0154	3.377
Succinic acid	10	0.0755	$4.6065x10^{-3}$	6.101
	30	0.2391	0.012	5.019
	50	0.3875	0.01395	3.600

Table 3: LOD and LOQ

S.No.	Co-Crystals	LOD	LOQ
1.	Spiranolactone-Saccharin co-crystals	0.0968	0.302
2.	Spiranolactone-Tartaric Acid co-crystals	0.1016	0.3173
3.	Spiranolactone-Succinic Acid co-crystals	0.1013	0.3162

Table 4: Accuracy studies

S.No.	Levels	Spiranolactone-Saccharin co-crystals	Spiranolactone-Tartaric Acid co-crystals	Spiranolactone-Succinic Acid co-crystals
1.	50%	107.54%	100.20%	109.10%
2.	100%	91.62%	76.80%	44.19%
3.	150%	74.07%	51.86%	105.12%

From the monograph, the characterisation of Spiranolactone by UV, FTIR and Melting point studies demonstrated that spiranolactoneis in pure form. From the FTIR and Melting point it can conclude that saccharin, tartaric acid and succinic acid samples are in pure form and it was selected for further investigations.

Analytical method development was done based on changing the mobile phase concentration and detection wavelength. Selection of mobile phase was done based on solubility studies of Spiranolactone and tartaric acid. Various mobile phases were tried in the UV to achieve a good peak response. From the various mobile phase trials, methanol was selected.

Validation of the developed UV method was done as per the ICH guidelines Q2(R1). The validation parameters such as linearity, LOD, LOQ, precision and accuracy were evaluated. Linearity and range of the methods were analysed by preparing calibration curves using different concentrations range of standard Spiranolactone-Succinic acid(10-50µg/ml) at 245nm. The calibration curve was plotted using peak area and concentration of the standard solutions. It was concluded that the perfect linearity was observed between the concentration and peak area and the concentration range from 10-50µg/ml. The results revealed that linear regression equation for spiranolactone-succinic acid was Y=0.006x and co-relation co-efficient (R^2) value 0.983. Precision studies were carried out and the mean, standard deviation (SD) were calculated and found to be within the limit. The results revealed that percentage co-efficient of variation (%RSD) was found to be >2%. Accuracy is reported as % recovery of the analysed concentration. The results indicate that the recovery of spiranolactone-saccharin was consistent at all levels and the percentage recovery for spiranolactone-saccharin was in between 40-110%. [9,10]

CONCLUSION

The design of new multicomponent crystal phases of Spiranolactone with desired physicochemical properties by applying crystal engineering technique. Capability to design new multicomponent crystal structures will depend mostly on supramolecular chemistry. The development of new supramolecular complexes, co-crystal and polymorphs of drugs by crystal engineering is becoming progressively more important as an alternative to salt formation, mainly for neutral or weakly ionisable compounds.

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