ORIGINAL ARTICLE

Drug-excipient Interaction Study for Apple Cider Vinegar with 20 Potential Excipients using Modern Analytical Techniques

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Abstract

Aim: To evaluate possible interaction between apple cider vinegar (ACV) which is the most frequent used active ingredient of diet pills with 20 different excipients. **Materials and Methods:** The pure drug ACV and drug-excipient powder mixtures which were stored under the condition of 40°C and 75% relative humidity for a period of 21-day and interaction were seen by using modern instrumental technique high-performance liquid chromatography, Fourier transmission infrared and differential scanning calorimetry. To investigate drug-excipient interaction and for selection of suitable excipient for diet pill a comparative study was done and each excipient was selected for formulation development only if it showed compatibility results by at least two instrumental techniques. **Results and Discussion:** The drug-excipient compatibility study revealed possible interaction between ACV and seven excipients out of 20 different excipients chosen. The excipient where interactions observed with active pharmaceutical ingredient by more than two techniques are magnesium stearate, polyethylene glycol-4000, CARBOPOL, crosspovidone, stearic acid, ethyl cellulose and colloidal silica which could be considered as not suitable for the development of formulation. **Conclusion:** Study indicates that what are the excipients which can be used for making pill of ACV a useful active ingredient as a diet supplement and antiobesity formulation.

Key words: Apple cider vinegar, diet formulation, drug-excipient interaction

INTRODUCTION

Excipients are usually biologically inactive, can stabilize and/or destabilize drug products. The same cannot be said from a chemical perspective. Incompatibility between drugs and excipients can alter stability and bioavailability of drugs thereby affecting the activity, safety and/or efficacy of a drug. The objective of drug/excipient compatibility considerations and practical studies is to delineate, as quickly as possible, real and possible interactions between potential formulation excipient and the active pharmaceutical ingredient (API).[1] This is an important risk reduction exercise early in formulation development. Drug-excipient studies are an important foundation tool early in the development of drug products. DECS data is essential for investigational new drug submission and drug approval process. Physical interactions can affect the rate of dissolution, uniformity of dose or ease of administration. Excipients may have functional groups that interact directly with active pharmaceutical ingredients. Alternatively, they may contain impurities or residues, or form degradation products that in turn cause decomposition of the drug substance.[2-5]

Apple cider vinegar (ACV) chemically is $C_2H_4O_2$ meaning it has three hydrogen atoms attached to one of the carbon atoms, one oxygen atom, and an oxygen-hydrogen atom attached to each other. The Fourier transmission infrared (FT-IR) spectrum of pure ACV showed an absorption band at 3558.42-3074.32cm^{-1}, assigned to the O-H stretching vibration. The chemical structure ACV shows the functional groups OH, C=O, C-O-H, and C-O which shows the peaks at 3558.42-3074.32 cm^{-1}, 1625 cm^{-1}, 1460 cm^{-1} and 1269 cm^{-1} in the IR spectra. Other functional peaks are also there but the therapeutic activity of this drug is due to the above 4 peaks.

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Being new to the formulation development world ACV has no reported excipient interaction results available in the literature. Hence, the aim of this study was to develop a suitable preformulation paradiagram based on which suitable excipients can be used with ACV formulations. This ingredient was being used to fight obesity and diabetes since a long time in various nations but not as a pharmaceutical formulation rather in an inconsistent form. But in the present scenario, many pharmaceuticals have developed simple diet pills containing ACV as ingredients and booming the market with such drugs but to make these pills more effective suitable excipient should be incorporated into the formulation for which this study will surely be beneficial. To avoid undesirable outcomes, selection of right excipient for ones formulation and guarantee its quality.[6]

MATERIALS AND METHODS

Materials

ACV was procured from Zim laboratories Nagpur. Excipients which were procured from B. R. Nahata College of Pharmacy, Microcrystalline cellulose, magnesium stearate, starch, sodium lauryl sulfate, polyvinylpyrrolidone (PVP)-K25, hydroxypropyl methylcellulose (HPMC), carbopol, talc, sodium carboxymethylcellulose, acacia, tragacanth, ethyl cellulose, and colloidal silica were supplied by SD Fine Chemicals Ltd., Lactose monohydrate, polyethylene glycol-4000 (PEG-4000) and crosspovidone were supplied by Merck Pvt. Ltd. Cellulose acetate was supplied by Nutan Gujarat Ind. States. Stearic acid was supplied by Loba Chemicals, Cross carmellose National Chemicals, Vadodara and Eudragit-RS were supplied by Ozone International. All other chemicals were obtained commercially as high-performance liquid chromatography (HPLC) or analytical grade reagents.

Analytical methods

All the samples of drug-excipient blends in ratio 1:1 are kept for 3 weeks at 40°C and 75% relative humidity storage conditions. After the lapse of 3 weeks, the samples were physically observed. It was then assayed by FT-IR, HPLC, and differential scanning calorimetry (DSC). Whenever feasible, the degradation products may be identified by mass spectroscopy, nuclear magnetic resonance (NMR) or other relevant analytical techniques. To determine solid state stability profile weighed samples were placed in an open screw cap vials and exposed directly to a variety of temperature, humidity and light intensities.[5,7]

DSC

DSC experiments were carried out with Shimadzu DSC-60 thermal analyzer.[8] Samples of about 2 mg were weighed in pierced AL pans and scanned under static air over a temperature range of 30-100°C and 180-250°C for ACV and gymnemic acid respectively at a heating rate of 10°C/min, the thermograms were reviewed for evidence of any interaction. Enthalpy calculations were completed using TA-60 software.

FT-IR

FT-IR spectra were recorded on a Shimadzu 8400 S using KBr discs in the range of 4000/400 cm^{-1}. The spectrum was a mean of 10 scans on the same sample.

High-performance liquid chromatography (HPLC)

Chromatograms were recorded using in house developed HPLC method.[9,10] For sample preparation, 2 mL of methanol was added into each vial. The mixture was vortexed and transferred to 100 mL volumetric flasks. Vials were rinsed twice with methanol, and the volume made up. The samples were centrifuged and the supernatant was filtered through a 0.45 µm nylon membrane filter. After appropriate, dilution samples were analyzed using a Waters 600 pump based HPLC system equipped with waters quaternary pump, waters manual injector, waters on-line degasser AF, CTO-10 AS VP column oven (at 28°C) and waters 2998 photodiode array detector (detection at 240 nm) was used. Chromatographic separation was performed on a lichrosphere (R) RP-18 e, with mobile phase acetonitrile: Buffer (pH2.6; 0.1% sulfuric acid) (90:10, V/V flow rate 0.5 mL/min.

RESULTS

Drug-excipient interaction study is an important paradiagram in the development of formulation. Mainly, the excipient compatibility of a drug is determined by instrumental techniques such as FT-IR, DSC, HPTLC, and HPLC. The DECS study involves observation of parameters such as change in peak, appearance of new peaks, peak merging, change in melting point, elimination of endothermic peaks, change in peak shape, onset of peak, and appearance of degradant (unknown peaks). Based on observation of these parameters drug excipient compatibility is obtained.[7] FT-IR interpretation results of ACV and with different excipient are shown in Figures 1-10. Study of the thermal behavior of ACV as shown in Figure 11 and its binary mixtures by DSC had revealed drug-excipient interaction between some of the excipients and drug. Interaction between ACV and excipient in its binary mixture lead to shifts in the characteristic melting point of pure drug in some of the binary mixtures shown in Figures 12 and 13.

HPLC analysis of ACV and its binary mixtures using mobile phase - Acn: Buffer (H_2SO_4-0.1% - PH-2.6) 90:10, flow rate - 0.5 ml/min, wavelength - 240 nm revealed the drug excipient interaction between some of the excipients and

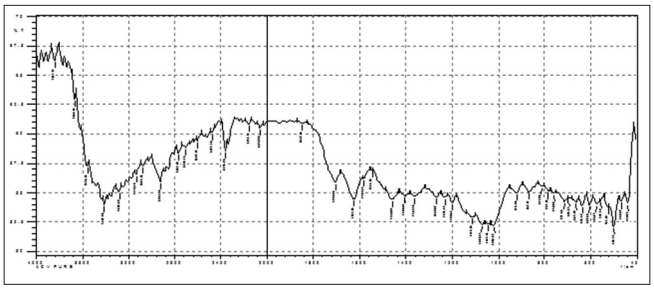

Figure 1: Fourier transmission infrared spectra of apple cider vinegar

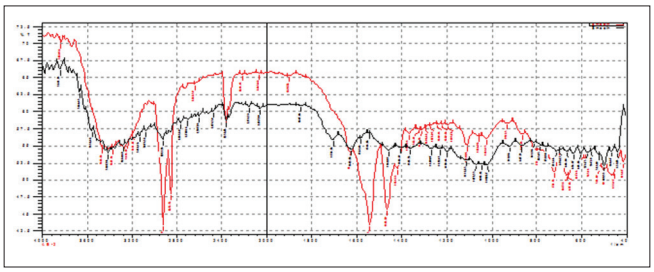

Figure 2: Fourier transmission infrared overlay spectra of apple cider vinegar+magnesium stearate

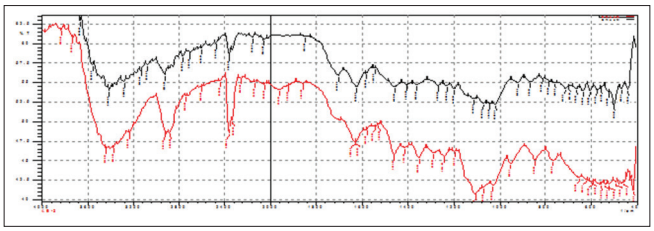

Figure 3: Fourier transmission infrared overlay spectra of apple cider vinegar+polyethylene glycol-4000

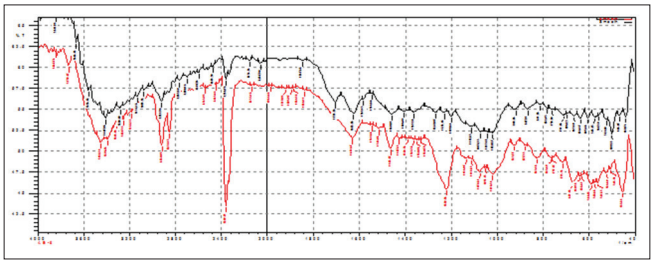

Figure 4: Fourier transmission infrared overlay spectra of apple cider vinegar+sodium lauryl sulfate

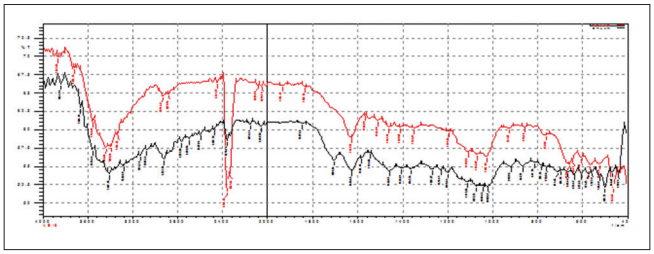

Figure 5: Fourier transmission infrared overlay spectra of apple cider vinegar+hydroxypropyl methylcellulose

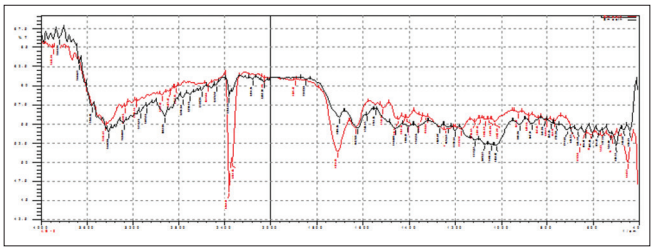

Figure 6: Fourier transmission infrared overlay spectra of apple cider vinegar+carbopol

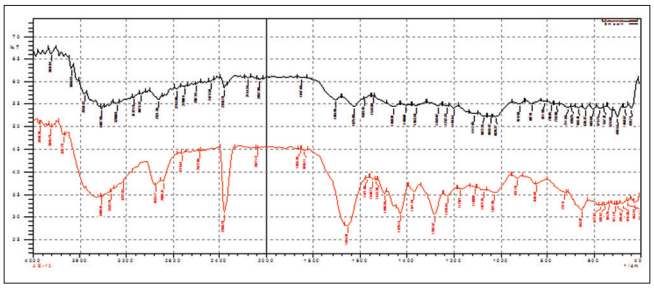

Figure 7: Fourier transmission infrared overlay spectra of apple cider vinegar+ crosspovidone

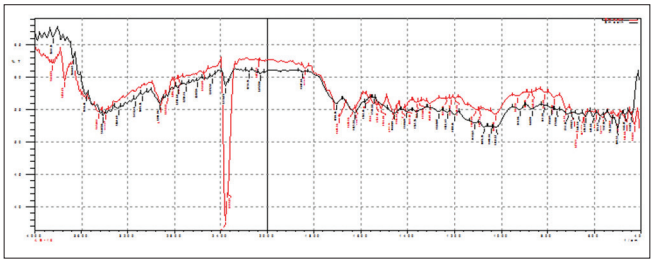

Figure 8: Fourier transmission infrared overlay spectra of apple cider vinegar+stearic acid

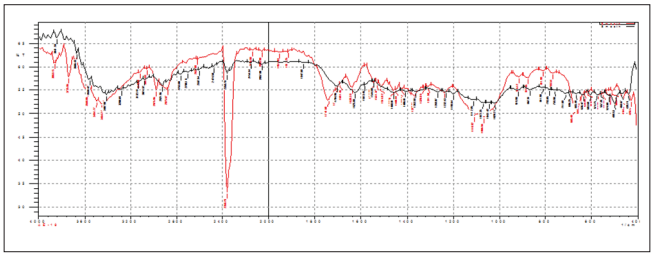

Figure 9: Fourier transmission infrared overlay spectra of apple cider vinegar+ethyl cellulose

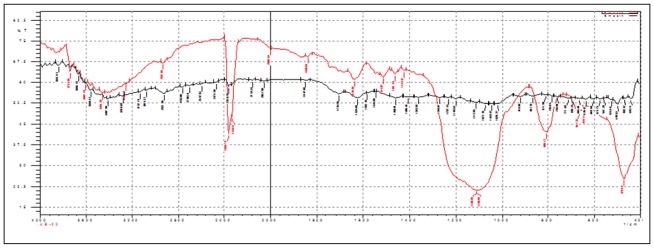

Figure 10: Fourier transmission infrared overlay spectra of apple cider vinegar + colloidal silica

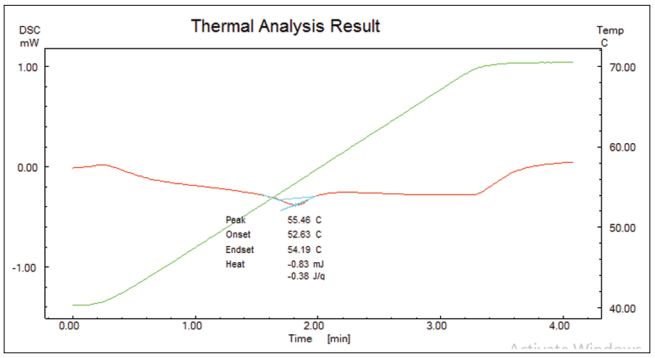

Figure 11: Differential scanning calorimetry thermogram of pure apple cider vinegar

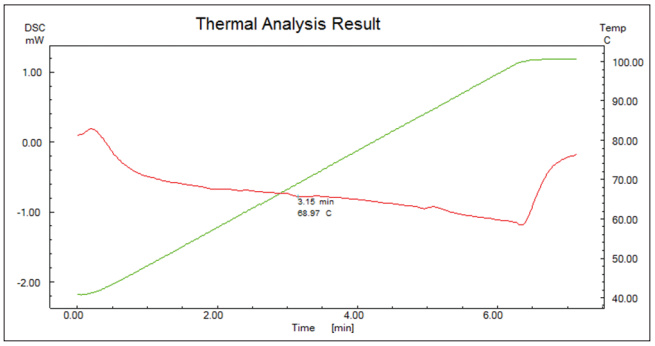

Figure 12: Differential scanning calorimetry thermogram for binary mixture of apple cider vinegar and ethyl cellulose

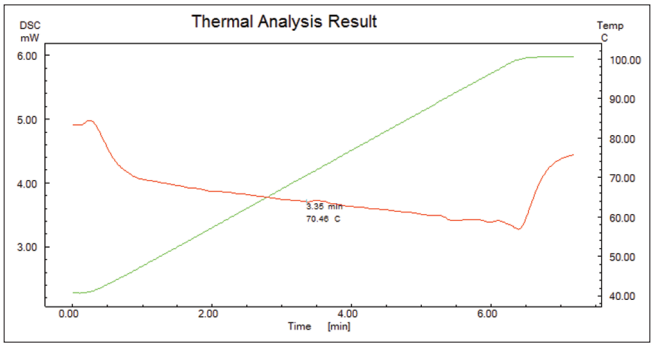

Figure 13: Differential scanning calorimetry thermogram for binary mixture of apple cider vinegar and colloidal silica

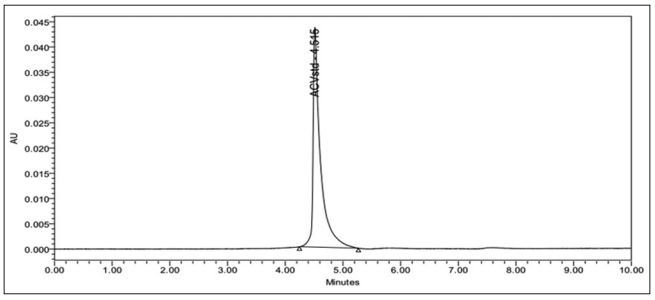

Figure 14: High-performance liquid chromatography chromatogram of pure apple cider vinegar

drug. Interaction between ACV and excipient in its binary mixture lead to shifts in the retention time (RT) of pure drug,

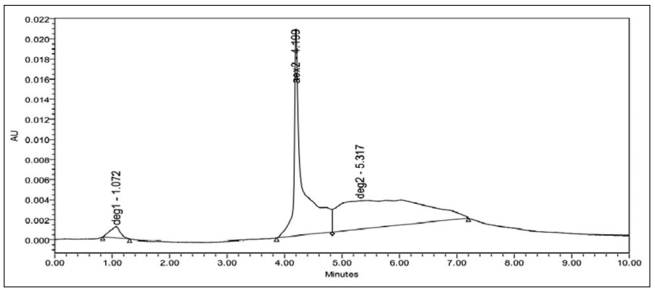

Figure 15: High-performance liquid chromatography chromatogram for binary mixture of apple cider vinegar and magnesium stearate

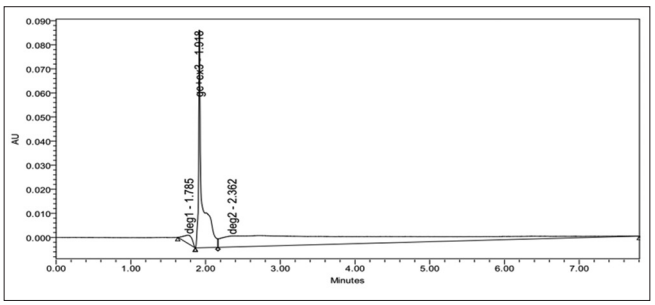

Figure 16: High-performance liquid chromatography chromatogram for binary mixture of apple cider vinegar and polyethylene glycol-4000

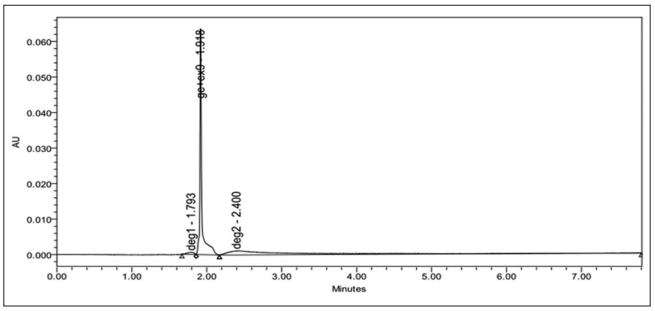

Figure 17: High-performance liquid chromatography chromatogram for binary mixture of apple cider vinegar and carbopol

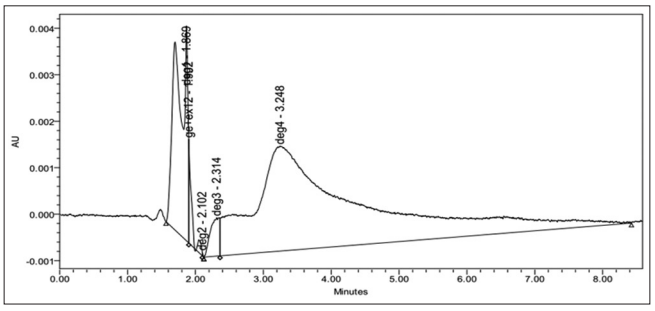

Figure 18: High-performance liquid chromatography chromatogram for binary mixture of apple cider vinegar and crosspovidone

change in percentage peak area of drug and appearance of degradant (unknown) peaks in some of the binary mixtures were shown in Figures 14-21. Table 1 shows summary of

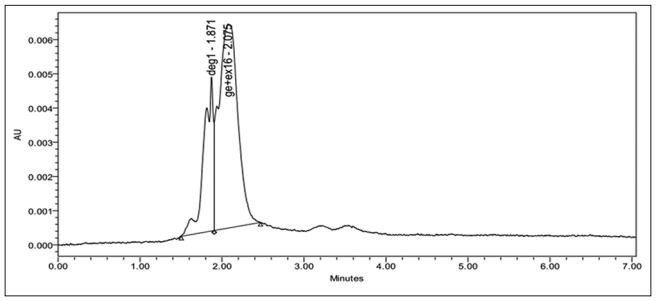

Figure 19: High-performance liquid chromatography chromatogram for binary mixture of apple cider vinegar and stearic acid

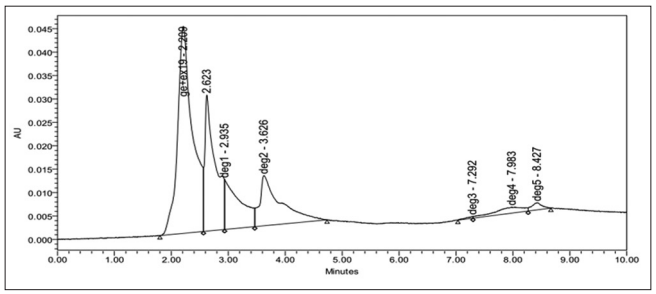

Figure 20: High-performance liquid chromatography chromatogram for binary mixture of apple cider vinegar and ethyl cellulose

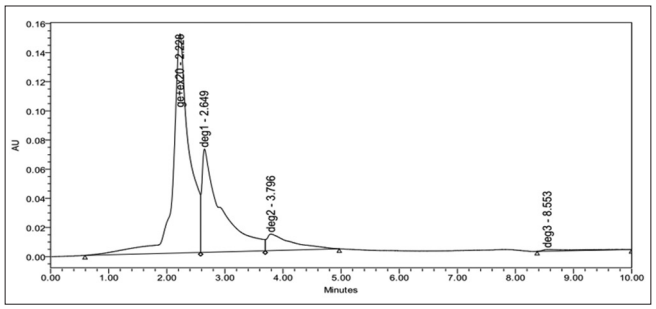

Figure 21: High-performance liquid chromatography chromatogram for binary mixture of apple cider vinegar and colloidal silica

FTIR, DSC, and HPLC results of pure drug and effect on it after mixing with different excipient.

DISCUSSION

FT-IR interpretation results of ACV from Figure 1 and its binary mixtures lead to suspicion of drug-excipient interaction between some of the excipients and drug. Interaction between ACV and excipient in its binary mixture lead to shifts in the characteristic peaks of pure drug or merging of characteristic peaks in some of the binary mixtures. The excipients where interactions were observed are magnesium Stearate where multiple peaks merged and characteristic C=O stretching was obtained at 1542 cm^{-1} instead of 1625 cm^{-1}, and C-O stretching at 1269 cm^{-1} was obtained as merged.

Table 1: Summary of interpretation results obtained by three techniques for drug and its binary mixtures with 20 excipients

Results	Pure	Interaction	Interaction	No interaction	No interaction	Interaction	Interaction	Interaction	Interaction	Interaction
HPLC										
DP	0	2	>3	0	1	1	>3	2	2	4
% Area	100	40	43.69	100	97.70	51	69.72	90.13	48.77	73.45
RT	4.515	4.199	4.277	4.492	4.487	4.300	4.312	4.214	4.161	4.687
DSC										
Time	1.96	1.97	1.89	1.97	1.97	1.97	1.97	1.95	3.15	3.35
T_m/°C	56.46	57.09	55.77	56.62	57.33	57.45	57.34	57.74	68.97	70.46
FT-IR										
$C\text{-}O_{stretching}$	1269	1110	1352	1220	-	1242	1226	1215	1238	-
$C\text{-}O\text{-}H_{stretching}$	1460	1465	1461	1463	1461	1448	1429	1461	1460	1461
$C{=}O_{stretching}$	1625	1542	1633	1631	1627	1633	1654	1693	1741	1639
$OH_{stretching}$	3558-3074	3600-3100	3600-3100	3560-3100	3600-3200	3600-3100	3600-3040	3600-3100	3650-3120	3660-3150
Sample code	ACV	AE2	AE3	AE6	AE8	AE9	AE12	AE16	AE19	AE20

AE-ACV+excipient, T_m/°C: Temperature in degree celcius, RT: Retention time, DP: Degradant peak, % Area: Percentage area of peak, ACV: Apple cider vinegar

In Figure 2 peak at 1110 cm^{-1} for PEG-4000 where characteristic C-O stretching was seen at 1352 cm^{-1} instead of 1269 cm^{-1} and O-H_{oop} bending 952 cm^{-1} instead of 919 cm^{-1} as shown in Figure 3, sodium lauryl sulfate where characteristic C-O stretching was seen at 1220 cm^{-1} instead of 1269 cm^{-1} as shown in Figure 4, HPMC where characteristic C-O stretching at 1269 cm^{-1} was not found as shown in Figure 5, carbopol where C-H stretching was merged with other peaks and a diffused peak was seen at 2943 cm^{-1} instead of a sharp peak at 2920 cm^{-1} and C=O sharp carbonyl peak at 1625 cm^{-1} was found merged with another peak seen at 1633 cm^{-1} as shown in Figure 6, crosspovidone.

Where C-H stretching showed broad peak at 2943 cm^{-1} instead of sharp peak at 2920 cm^{-1} and C=O sharp carbonyl peak at 1625 cm^{-1} was found as a broad peak at 1654 cm^{-1} as shown in Figure 7, stearic acid where characteristic C=O stretching was obtained at 1695 cm^{-1} instead of at 1625 cm^{-1} and C-O stretching at 1269 cm^{-1} was obtained as fused peak at 1215 cm^{-1} as shown in Figure 8, ethyl cellulose where C-H stretching has shown.

Sharp peak at 2975 cm^{-1} instead of sharp peak at 2920 cm^{-1} as shown in Figure 9, colloidal silica where C-H stretching appeared as fused peak at 2931 cm^{-1} instead of sharp peak at 2920 cm^{-1} and characteristic C-O stretching and O-H_{oop} bending were absent as shown in Figure 10.

Study of thermal behavior of ACV as shown in Figure 11 and its binary mixtures by DSC The physical mixtures where interactions (shifts of endothermic peak) were observed are Ethyl Cellulose where the shift in melting point was observed from standard 56.46°C to 68.97°C as shown in Figure 12 and colloidal silica where the shift in melting point was observed from standard 56.46°C to 70.46°C shown in Figure 13.

Chromatogram of ACV pure can be seen in Figure 14 and the excipients where interactions (shifts) and degradant (unknown) peaks were observed are magnesium stearate where shift in RT was observed from 4.51 to 4.19 and two unknown peaks were also observed followed by decrease in % peak area to 40% as seen in Figure 15, PEG-4000 where shift in RT was observed from 4.51 to 4.27 and more than three unknown peaks were also observed followed by decrease in % peak area to 43% as seen in Figure 16, carbopol where shift in RT was observed from 4.51 to 4.3 and one unknown peak was also observed followed by decrease in % peak area to 51% as seen in Figure 17.

Crosspovidone where shift in RT was observed from 4.51 to 4.3 and more than three unknown peaks were also observed followed by decrease in % peak area to 69.7% as seen in Figure 18, stearic acid where shift in RT was observed from 4.51 to 4.2 and two unknown peaks were also observed followed by decrease in % peak area to 90% as seen in Figure 19, ethyl cellulose where shift in RT was observed from 4.51 to 4.16 and two unknown peaks were also observed followed by decrease in % peak area to 48.7% as seen in Figure 20 and colloidal silica where shift in RT was observed from 4.51 to 4.7 and more than three unknown peaks were also observed followed by decrease in % peak area to 73.4% as seen in Figure 21.

CONCLUSION

With the advent of time obesity and its associated diseases such as diabetes and hypertension have told the death ratio to an unpredicted height, which has lead to the development and boom in the market of diet formulations. However, there is not much data available for basic preformulation parameters of such formulations hence present study has involved ACV an active ingredient of regular diet formulations for DECS study with 20 excipients. Moreover, the study results were concluded based on data obtained from FT-IR, DSC, and HPLC which can be seen in Table 1. The excipient where interactions observed with API by more than two techniques are magnesium stearate, PEG-4000, CARBOPOL, crosspovidone, stearic acid, ethyl cellulose and colloidal silica which could be considered as not suitable for development of formulation whereas the excipients where there is no interaction or interaction results are not supported by more than one technique are MCC, starch, lactose monohydrate, SLS, PVP-K25, HPMC, talc, cellulose acetate, sodium CMC, acacia, tragacanth, cross carmelose and eudragit-RS which could be used for further development of suitable diet formulations. Further confirmations can be obtained by mass and NMR spectroscopic study.

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