ORIGINAL ARTICLE

Incompatibility studies by high performance thin-layer chromatography: In case of curcumin

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Curcumin[1,7-bis (4-hydroxy-3-methoxy-phenyl) hepta-1, 6-diene-3, 5-dione] is one of the component present in the turmeric. Curcumin has been in use for its medicinal benefits since centuries and its therapeutic potential is continuously explored through various researchers throughout the world. To investigate the interaction of curcumin with commonly used excipients such as microcrystalline cellulose, starch, colloidal silica, talc, ascorbic acid, lactose, ethyl cellulose (EC), sodium carboxymethylcellulose (Na-CMC), hydroxyl propyl methyl cellulose and magnesium stearate. High performance thin-layer chromatography (HPTLC) is commonly used technique for the determination of phytoconstituents, but its application in incompatibility studies is still not investigated. Thus, we initiated our study with HPTLC followed by Fourier transform infrared and differential scanning calorimetry. Since interaction of curcumin with ascorbic acid, EC, Na-CMC and Mg-stearate confirmed by all three techniques these four excipients should be avoided during the formulation development of curcumin. The presented study also establishes HPTLC's usefulness in such interaction studies.

Key words: *Curcumin and incompatibility study, differential scanning calorimetry, fourier transform infrared, high performance thin-layer chromatography*

INTRODUCTION

Curcumin 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-2,5-dione is a yellow colored phenolic pigment obtained from powdered rhizome of *Curcumalonga* Linn.(Family: Zinziberaceae), from ancient it was being used for relieving the pain and inflammations in ancient times in traditional medicine.[1] Curcumin, a yellow pigment present in the Indian spice turmeric (associated with curry powder), has been linked with suppression of inflammation; angiogenesis; tumorigenesis; diabetes; diseases of the cardiovascular, pulmonary, and neurological systems, of skin, and of liver; loss of bone and muscle; depression; chronic fatigue; and neuropathic pain.[2] Curcumin, an Indian spice with antioxidant, anti-inflammatory and anti-cancer properties, has shown promise both as a potential chemopreventive agent as well as an ovel adjuvant treatment for head and neck malignancies.[3] Therefore, it may have potential to develop in to modern drug.

Most excipients haven't direct pharmacological action but they canal so give rise to in advert entorin intended effect such as increase degradation of drug. Physical and a chemical interaction between drug and excipients can affect the chemical nature, stability, bioavailability of product, therapeutics efficacy and safety.[4-6]

Thermoanalytical techniques measure the changes in physical or chemical properties of the sample as a function of temperature. There are many possible applications in pharmaceutical industry, for example, identification, characterization of active and inactive ingredients, routine analysis, quality control and stability study.[7-10] The main benefit of differential scanning calorimetry (DSC) is its availability to quickly screen potential excipients for incompatibility derived from the shifting, appearance, disappearance of endothermic/exothermic peak or variation in the corresponding ⊗H (Enthalpy of transition).[11]

The identification of possible incompatibilities between drug and excipients is one of the basic tasks to be dealt with in a pre-formulation laboratory. In this sense,

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devising a quick and accurate method to test and select the best candidates for stable dosage forms would constitute are break through in the pre-formulation pharmacy.[12] DSC is one of the well-established techniques in detection of incompatibility in drug/excipient. DSC has now become first choice in pharmaceutical industry for compatibility study. Fourier transform infrared (FTIR) spectroscopy is also used to confirm any type of physical interaction with drug and excipient.[13] The main benefit of DSC is its availability to quickly screen potential excipients for incompatibility derived from the shifting, appearance, disappearance of endothermic/exothermic peak or variation in the corresponding ΔH (enthalpy of transition).[14]

The usage of high performance thin-layer chromatography (HPTLC) is well appreciated and accepted all over the world. It is due to its numerous advantages, for example, it is the only chromatographic method offering the option of presenting the results as an image.[15] The modern HPTLC technique, combined with automated sample application and densitometric scanning, is sensitive and completely reliable, suitable for use in qualitative and quantitative analysis.[16] Other advantages includes implicity, low costs, parallel analysis of samples, high sample capacity, rapidly obtained results, and possibility of multiple detection.[17] This is our opinion that there will be some change in the chromatogram if there is any interaction between drug and excipient. Following study was under taken to establish incompatibility studies as another application of HPTLC technique. Data of incompatibility studies were matched with FTIR and DSC. In the presented study HPTLC, FTIR and DSC were successfully used to established compatibility of curcumin with various pharmaceutical excipients for development of suitable formulation.

EXPERIMENTAL

Materials

Curcumin and various excipients viz., micro crystalline cellulose (MCC), starch, colloidal silica, talc, ascorbic acid, lactose, ethyl cellulose (EC), sodium carboxy methyl cellulose (Na-CMC), hydroxyl propyl methyl cellulose (HPMC), and magnesium stearate were purchased from Sigma-Aldrich and Alfa-Aesar (USA) and used as such.

Methods

HPTLC chromatogram data's were performed on a CAMAG instrument to find out the incompatibility of the drug with excipients used in the formulation. HPTLC chromatogram of the drug and binary mixtures were obtained by using the composition of chloroform and methanol in the ratio of 9.7:0.3v/v as mobile phase on pre-coated silicagel F_{254} plates used as stationary phase.[18] The HPTLC analysis has been performed using pure curcumin and their binary mixtures (1:1massratio).

FTIR spectra of sample were measured in a Shimadzu spectrophotometer (8400S), in a scan range of 400-4000 cm^{-1} with an average of over 15 scans at a spectral resolution of 4 cm^{-1} in potassium bromide (KBr). A background spectrum was obtained for each experimental condition.

Compatibility studies of curcumin with various excipients carried out by Shimadzu DSC-60230 V. Sample was weight out and placed in a sealed aluminum pan and scanned from room temperature to 300°C with heating rate of 10°C/min. The DSC analysis has been performed using pure curcumin and their binary mixtures (1:1massratio).

RESULT AND DISCUSSION

In the present study an attempt as been made to drug excipients compatibility studies were carried out to check whether any compatibility related problems are associated between drug and excipients used in the formulations.

HPTLC result discussion

In HPTLC technique, retardation factor (Rf) value for the drug was around 0.53 with peak area of 28549.2. HPTLC studies revealed that the Rf values obtained for binary mixture containing MCC, starch, colloidal silica, talc, ascorbic acid, were around 0.52-0.53 [Figure 1 and Table 1].

The mixture containing MCC and starch showed slight decrease in Rf value as compare to pure curcumin of 0.52 and may be due to well-known hygroscopic nature of these component. There is as light decrease also in peak area of binary mixture as compare to curcumin. In EC and lactose Rf value was found to be increased as compare to pure drug (Rf: 0.58). With EC lowest peak area was found as compare to all binary mixtures, while the mixture containing Na-CMC, HPMC, Mg-stearate showed increase in Rf value as compare all other mixtures, but the peak area was decreased as compare to others.

Rf of the binary mixtures containing lactose, EC, Na-CMC, HPMC, Mg-stearate were shuffled and peak areas also decreased compared to pure curcumin [Figure 2]. Lowest peak area found in case of binary mixture containing EC of 3987 [Figure 3].

Those facts clearly indicate the suspicious interaction of curcumin with these excipients in binary mixture as compare to other one. This suspection is confirm/cross checked by further FTIR study and also by DSC.

FTIR result

FT-IR spectra of sample were measured in a Shimadzu (8400 S) spectrophotometer, in as can range of 400-4000 cm^{-1} with an average of over 15 scans at a spectral resolution of 4 cm^{-1} in KBr. Drug and various excipients were thoroughly mixed with KBr, compressed and the spectrum was obtained by placing the thin pellet in light path.

Table 1: HPTLC data of curcumin with different excipients

Excipients	Rf	Peak area	Change	Conclusion
Pure drug	0.53	28549.2		
MCC	0.52	18135.5	No change	No interaction
Starch	0.52	14613.3	No change	No interaction
Colloidal silica	0.53	19706.6	No change	No interaction
Talc	0.53	21126.4	No change	No interaction
Ascorbic acid	0.53	13738.4	No change	No interaction
Lactose	0.58	11428.6	Shift of RT and area decrease	Suspection
Ethyl cellulose	0.58	3987	Shift of RT and area decrease	Suspection
Na-CMC	0.59	7075.1	Shift of RT and area decrease	Suspection
HPMC	0.59	9045.2	Shift of RT and area decrease	Suspection
Mg-Sterate	0.59	8449.7	Shift of RT and area decrease	Suspection

HPTLC: High performance thin-layer chromatography, MCC: Micro crystalline cellulose, Na-CMC: Sodium carboxy methyl cellulose, HPMC: Hydroxy propyl methyl cellulose, RT: Real time

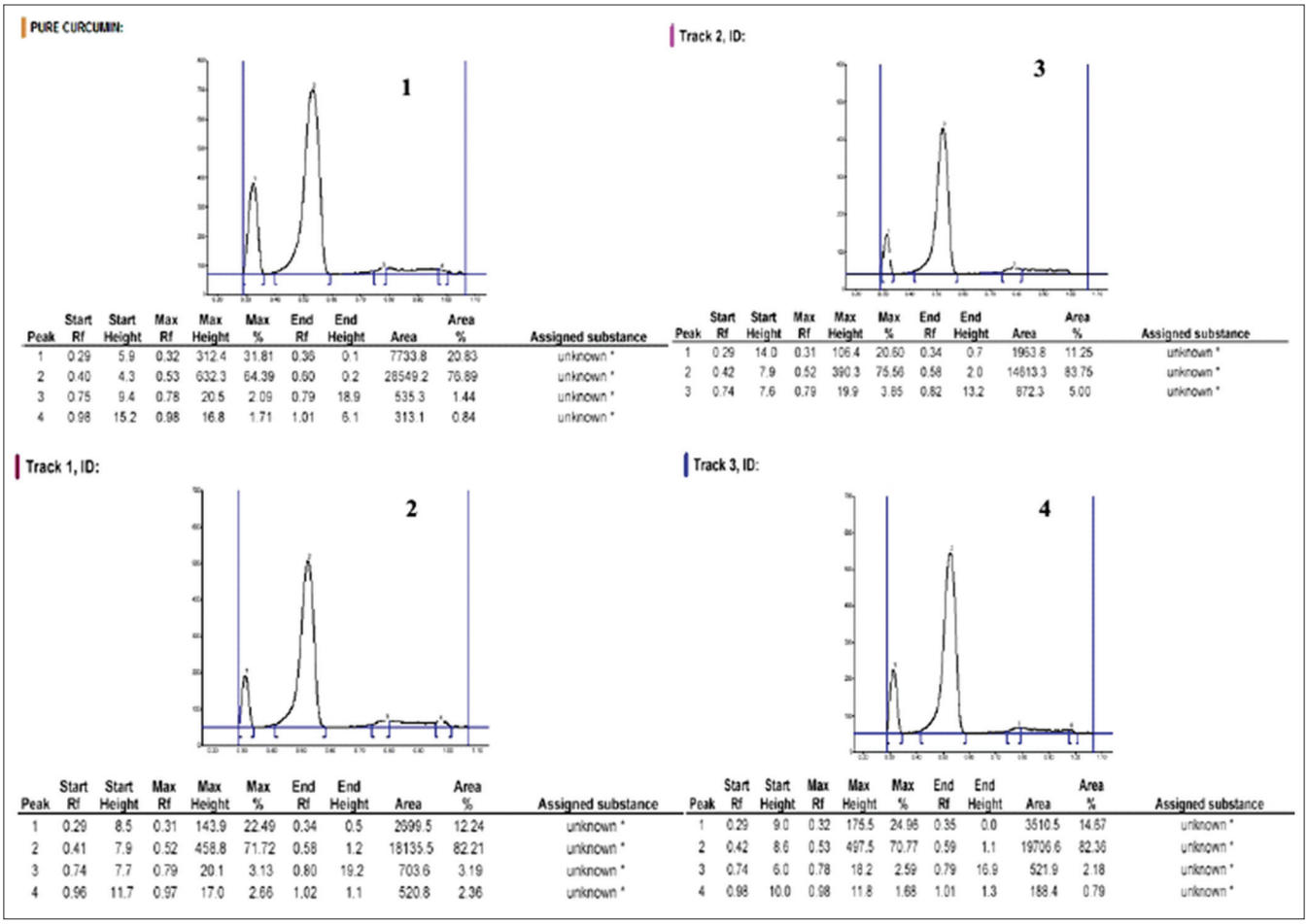

Figure 1: Chromatogram of pure curcumin (1), curcu + micro crystalline cellulose (2), curcu + starch (3), curcu + Coll.silica (4)

The FTIR spectra of curcumin show vibration of phenolic group appeared at 3504 cm^{-1}. The peak of C = C stretching in aromatic and allopathic appeared at 1610, 1560 cm^{-1}. Curcumin contains two carbonyl groups, showing the values around 1640 cm^{-1}. Tri substituted benzene give OOPB [out of plane bend] at 800 cm^{-1}. Infrared studies reveal that both characteristic bands around 1640 and 1610 cm^{-1} are present. While no new bands or shift in characteristic peaks appeared in case of binary mixture.

The FTIR spectra of binary mixture containing lactose [Figure 4] show alteration in C = O stretching value as compare to pure curcumin is of 1766 cm^{-1}. While in case of EC [Figure 5] C = O aromatic stretch value may rise upto highest as compare to all suspected binary mixture is of 1629 cm^{-1}. The Na-CMC [Figure 6] also showed high peak value of phenolic OH stretching of 3548 cm^{-1}. Is also showed highest peak value of 2972cm^{-1}. The Ascorbic acid [Figure 7] show lowest C = C aliphatic stretching (1506 cm^{-1}) The mixture

contains Mg-stearate showed alteration in peak value of C-H stretching (aromatic group) is of 2918 cm^{-1} which is the lowest one [Figure 8]. Details of IR spectra obtained is given under Table 2.

The result shown in FTIR Study is slightly similar to result coined in HPTLC. However, in FTIR spectra appearance of any new band/peak was not seen. Still there may be slight interaction. The result obtained by HPTC and FTIR were then finally cross checked by DSC study.

DSC result discussion

The DSC graph was carried out by sample weighed directly in the DSC aluminum pan and scanned from 40°C to 200°C at

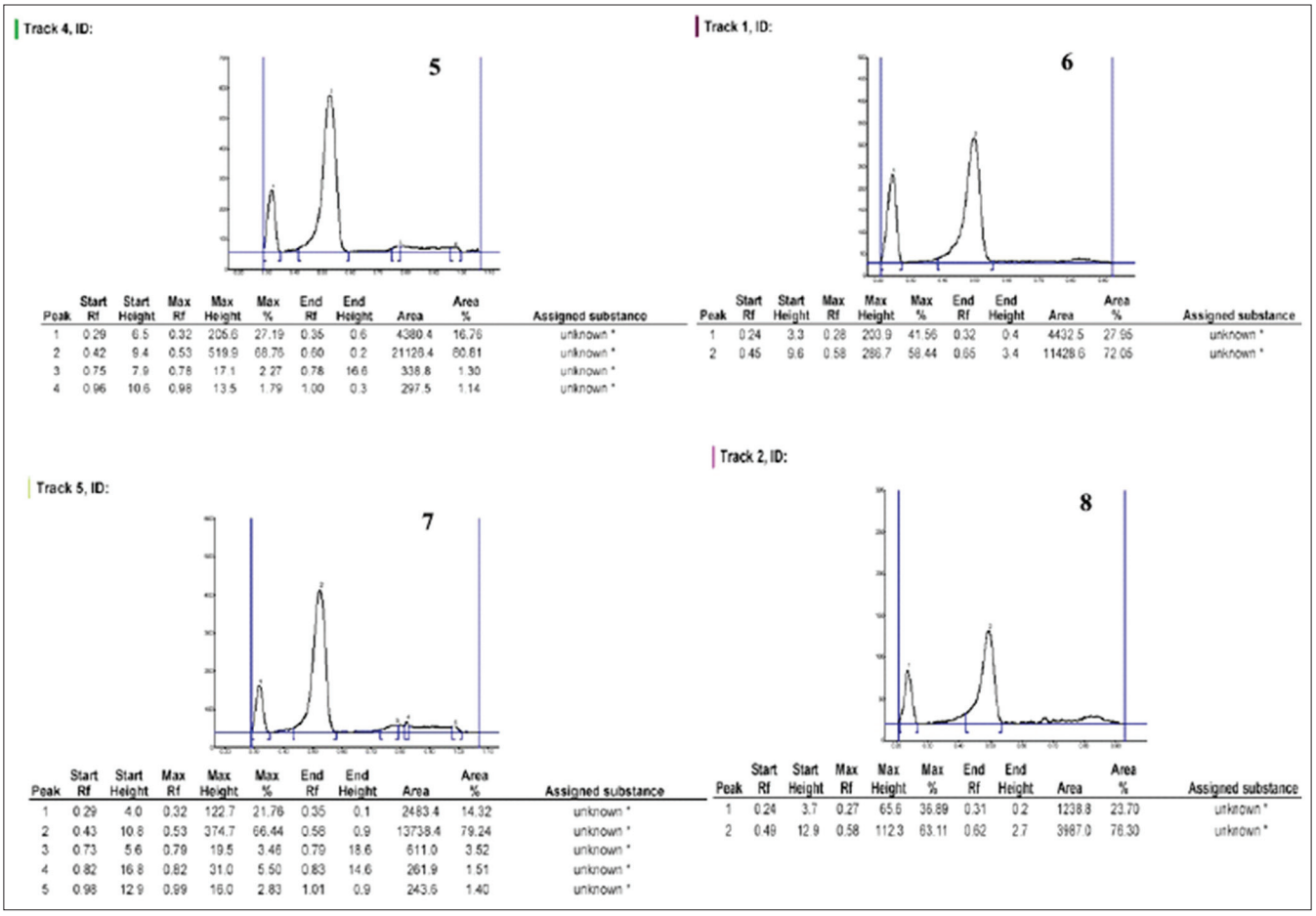

Figure 2: Chromatogram of curcu + talc (5), curcu + ascorbic acid (6), curcu + lactose (7), curcu + MM (8)

Table 2: FTIR-data of curcumin with different excipients

Functional group drug+mix	IR frequency of drug and its binary mixture with excepients (cm^{-1})						
	Phenolic-OH stretching	-C-H stretching	Aromatic overtone	C=O stretch	C=C aromatic	C=C aliphatic	Trisubstituted benzene (OOPB)
Curcumin	3504	2889	2100-1800	1640	1610	1560	800
MCC	3533	2888	2100-1800	1643	1620	1564	811
Starch	3520	2927	2100-1800	1627	1602	1562	811
Colloidal Silica	3540	2923	2100-1800	1697	1627	1510	811
Talc	3535	2927	2100-1800	1735	1629	1512	811
Ascorbic acid	3539	2987	2100-2800	1751	1627	1506	815
Lactose	3501	2900	2100-1800	1766	1627	1510	811
EC	3480	2937	2100-1800	1747	1629	1508	811
Na-CMC	3548	2972	2100-1800	1741	1627	1515	813
HPMC	3508	2873	2100-1800	1697	1600	1510	811
Mg-stearate	3524	2918	2100-1800	1741	1625	1510	811

OOPB: Out of plane bend, MCC: Micro crystalline cellulose, Na-CMC: Sodium carboxy methyl cellulose, HPMC: Hydroxy propyl methyl cellulose, FTIR: Fourier transform infrared, IR: Infrared, EC: Ethyl cellulose

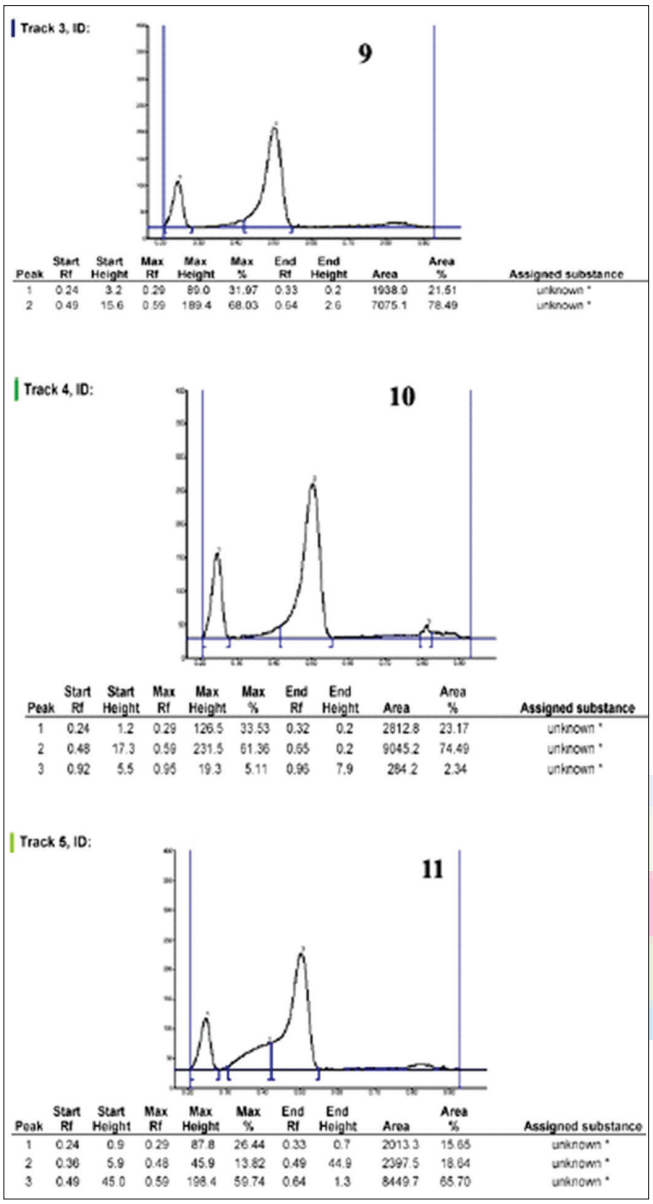

Figure 3: Chromatogram of curcu + Na-carboxy methyl cellulose (9), curcu + hydroxy propyl methyl cellulose (10), curcu + Mg-stearate (11)

the heating rate of 10°C/min under the N_2 atmosphere. The DSC curve of Tamsulosin may show a sharp end other mic peak [melting temperature (T_m)] at 177.76°C in 14.26 min (melting time) [Table 3].

DSC results revealed that the physical mixture of Curcumin with excipients showed superimposition of the thermograms. There is no considerable change observed in melting endotherm of binary mixture containing MCC, Starch, colloidal silica, talc, lactose, HPMC were shown in Table 3. The binary mixture containing ascorbic acid, lactose, Na-CMC, EC, Mg-stearate [Figures 9-11] were susceptible to be an interaction. Lowest melting temperature (T_m) found in case of binary mixture containing Mg-stearate is of 171.97°C [Figure 12]. Also lowest time required for melting was found in this case.

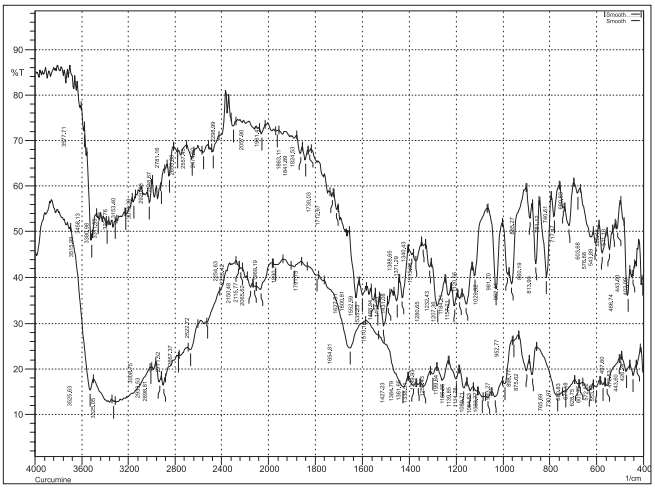

Figure 4: Infrared spectra of CU + lactose

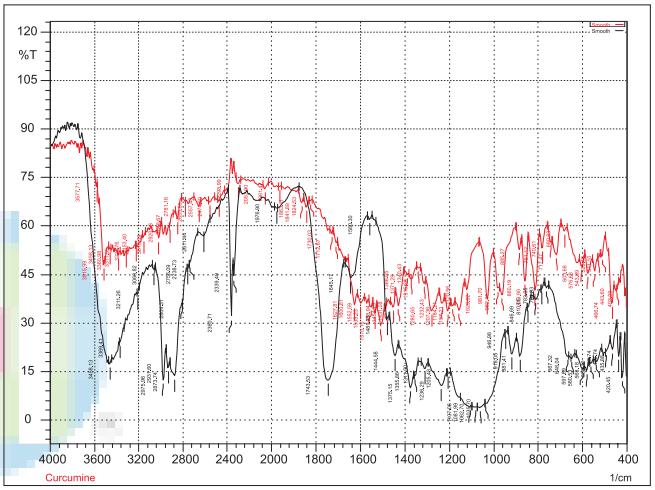

Figure 5: Infrared spectra of CU + EC

Table 3: DSC data of curcumin with different excipients

Excipients	T_m/°C	Time (min)	Change	Conclusion
Pure drug	177.76	14.26		
MCC	178.76	14.43	No	No interaction
Starch	178.51	14.39	No	No interaction
Colloidal silica	179.32	14.43	No	No interaction
Talc	178.22	14.36	No	No interaction
Ascorbic acid	176.55	14.17	Yes	Interaction
Lactose	179.37	14.50	No	Interaction
Ethyl cellulose	174.87	14.07	Yes	Interaction
Na-CMC	183.78	14.90	Yes	Interaction
HPMC	179.24	14.52	No	No interaction
Mg-Sterate	171.97	13.75	Yes	Interaction

MCC: Micro crystalline cellulose, Na-CMC: Sodium carboxy methyl cellulose, HPMC: Hydroxy propyl methyl cellulose, DSC: Differential scanning calorimetry

CONCLUSION

Curcumin is the principal curcuminoid and comprises approximately 2-5% of turmeric; it is responsible for the yellow color of the spice as well as the majority of turmeric's

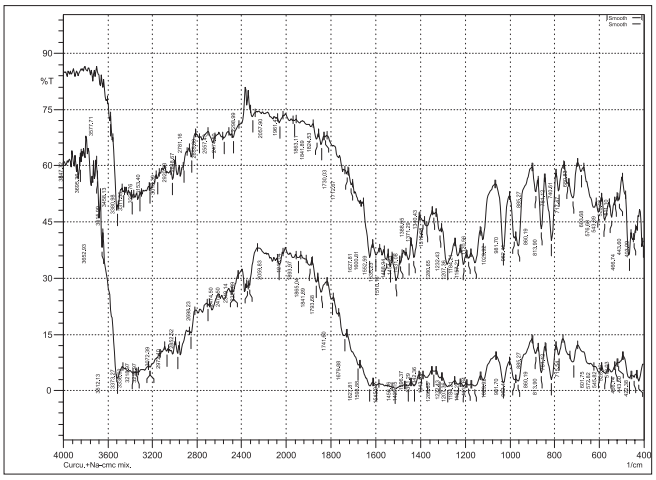

Figure 6: Infrared spectra of curcu + Na-carboxy methyl cellulose

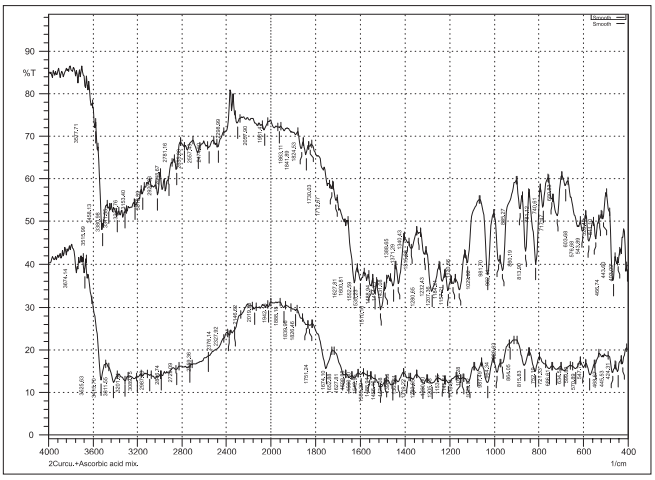

Figure 7: Infrared spectra of curcu + ascorbic acid

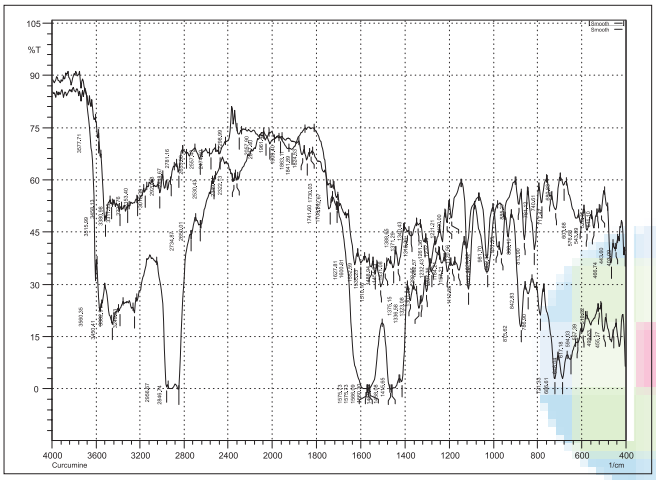

Figure 8: Infrared spectra of curcu + Mg-stearate

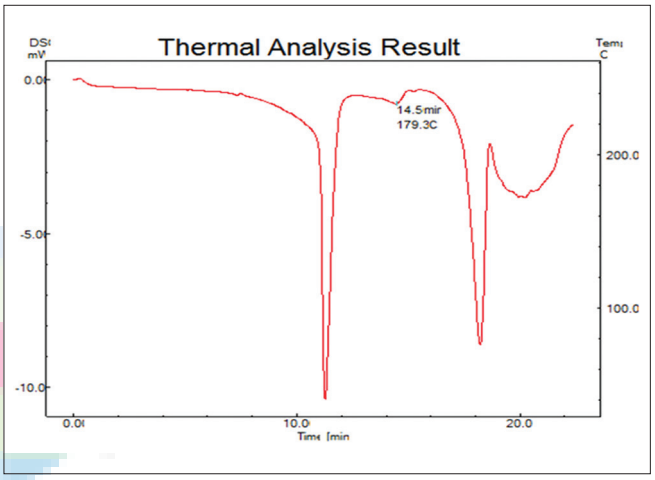

Figure 9: DSC curve of curcumin + lactosemix

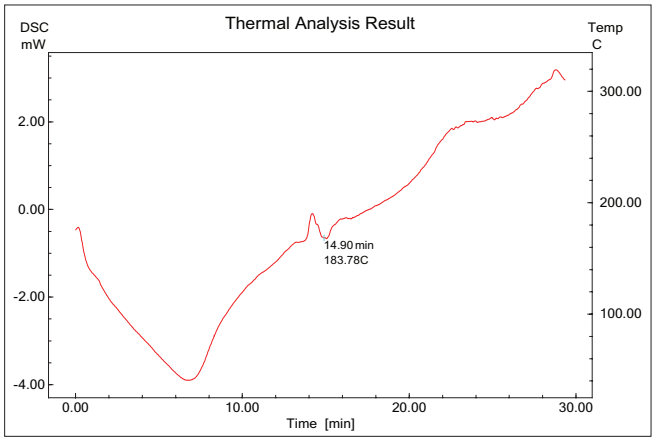

Figure 10: DSC curve of curcumin + Na-carboxy methyl cellulose mix

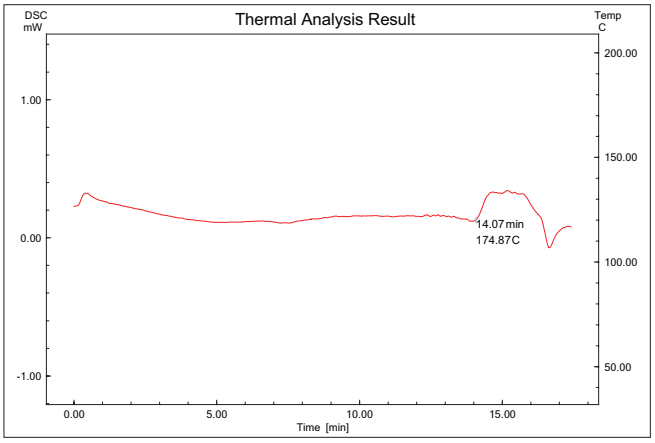

Figure 11: DSC curve of curcumin + ethyl cellulose mix

therapeutic effects.[3,19] Presented drug excipients interaction study of curcumin was also aimed to establish HPTLC as another technology can be used in this field. Interaction of curcumin with lactose, EC, Na-CMC, HPMC, Mg-stearate is well evident with HPTLC in binary mixtures due to change in Rf and peak area whereas, FTIR and DSC both shows interaction with ascorbic acid, lactose, EC, Na-CMC and Mg-stearate. We recommend HPTLC can be used as auxiliary technique for such kind of pre-formulation studies or can be used initially during development of analytical methods to give some idea about any interaction. Our recommendation is based on the fact that HPTLC shows interaction with four excipients ascorbic acid,

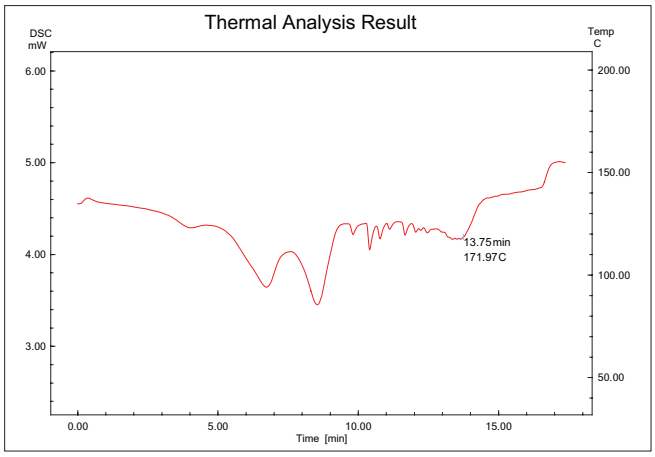

Figure 12: DSC curve of curcumin + Mg-stearate mix

EC, sodium CMC and Mg-stearate was also confirmed by both FTIR and DSC. Since interaction of curcumin with ascorbic acid, EC, Na-CMC and Mg-stearate confirmed by all three techniques we concluded that these four excipients should be avoided during formulation development of curcumin. Further studies using nuclear magnetic resonance is suggested to prove sufficient interaction.

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