ANALYTICAL METHODS FOR QUALITY CONTROL OF DIFFERENT BRANDS

Quality of drugs: Global issues

In recent years, the emergence of counterfeit medicines has become a serious problem on the national and international level. In most developed countries due to effective regulatory systems and control on pharma market, the incidence of counterfeit medicines is less as compared to the developing countries—especially in Asia where many counterfeit medicines are produced, and in countries like Africa, where poverty and loosed regulatory oversight make the existence counterfeit products easier (1).

A World Health Organization (WHO) survey conducted for 20 countries for the period of January 1999 to October 2000, in context of counterfeiting, found that 60% of counterfeit medicine comprised of poor countries and 40% of industrialized countries. A study conducted in South East Asia in 2001 revealed that 38% of 104 antimalarial drugs in the market (for sale) did not contain any active ingredients and had caused a number of deaths. In 2002, Glaxo SmithKline in the United States (though a developed country) discovered counterfeit bottles of Combivir tablets (lamivudine plus zidovudine) which actually contained another medicine, Ziagen (abacavirsulfate). In Cambodia, in the year 1999, minimum 30 people had died after taking counterfeit antimalarials prepared with sulphadoxine and pyrimethamine (an older, less effective antimalarial); these were sold as Artesunate (1).

The United States Food and Drug Administration have estimated that counterfeit drugs make up to a more than 10% of the global medicines' market and such counterfeiting is found in both industrialized and developing countries. It has been estimated that up to 25% of the medicines consumed in poor or developing countries are counterfeit or substandard (1).

According to the Pharmaceutical Security Institute report 2010, China, India, Paraguay, Pakistan and UK have been detected as the top five origin countries of counterfeit medicines. The report stated that China and India were the top origins of detected counterfeits in 2010 according to the Pharmaceutical Security Institute report 2010. The institute reported 2177 incidents globally for pharmaceutical crime out of which 875 incidences were from Asia (1, 2).

Quality of drugs: Indian scenario

According to the literature of Assochem, Delhi (National Capital Region) NCR has been detected as the biggest centre for counterfeits. Fake medicines' market occupies a big space in India's domestic drug market. Particularly the regions of Gurgaon, Faridabad and Noida have been reported in this context. The fake medicines constitute nearly one-third of all drugs sold in NCR. The counterfeit drugs constitute US$ 4.25 billion of the total US$ 1417 billion of domestic drugs market. If the counterfeit market grows at the current rate i.e. of 25%, it can cross US$ 10 billion mark by 2017. The counterfeiting was found in popular drugs like Crocin, Voveran, Betadine, injections of calcium and syrups like Cosavil (3, 4).

In general, poor quality drugs which may cause treatment failure are spurious/falsely-labeled/falsified/counterfeit (SFFC) medicines. WHO has defined SFFC drugs as "medicines that are deliberately and fraudulently mislabeled with respect to identity and/or source and also which may include products with correct ingredients or with the wrong ingredients, without active ingredients, with insufficient or too much active ingredient, or with fake packaging" (1). The Drugs and Cosmetics Act, 1940 also defines the misbranded, adulterated and spurious drugs under the sections 17, 17A and 17B respectively (5). The pharma market contains various brands for the same formulation; may it be contract manufacturing companies or those manufacturing generic products. Hence it becomes a crucial need to study the quality all these brands and to develop analytical techniques which could differentiate between genuine ones and the counterfeits.

The government and drug regulatory bodies of all countries have now started taking crucial steps for minimizing and hence the control of counterfeiting. Various analytical methods hence play very important role in the detection of the fake drug, though it is very difficult to analyze quality fakes. HPLC is quite efficient in analyzing substandard (low and high concentration) drugs whereas MS can provide information about foreign moieties present in the formulation. Qualitative detection or discrimination between the SFFC drugs is normally done by spectroscopic techniques such as Raman spectroscopy, near infrared spectroscopy (NIR) and Fourier transform infrared spectroscopy (FTIR). These methods are preferred because they are fast and need only a little or no preparation (6). X-ray fluorescence is also used for the

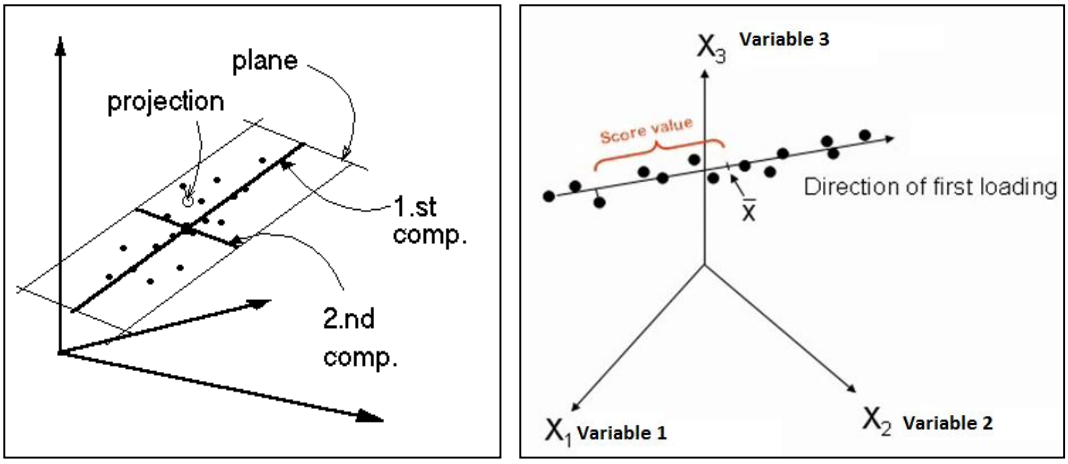
counterfeit identification (7). However the data of these techniques are found to be very vast and complicated and hence the use of statistical tool always aids in to make the analysis simpler. Thus the Exploratory data analysis is used for further simplification of the spectral data.

Exploratory data analysis (EDA) (8, 9, 10, 11)

The EDA uses the chemometric techniques such as Principal component analysis (PCA) and Hierarchical cluster analysis (HCA), which are designed to reduce large complex data sets to simplified and interpretable views. These views highlight the natural groupings in the data and show which variables most strongly influence those patterns. These techniques prove to be very powerful tool for discrimination and counterfeit study with respect to the original or reference sample. The PCA technique is combined with hierarchical cluster analysis to establish an automated approach for the discrimination between **different groups of brands or counterfeits and genuine products**.

PCA can compress a large data set (matrix) into a smaller space that is easy to overview. In PCA, a data matrix is constructed to extract meaningful information from multivariate data. The information obtained can be supervised and/or unsupervised classification or curve resolution. Data resolution means taking a look on data to find some highlighting or interesting phenomenon. As a result, outlier, clustering of objects and gradients between clusters may be detected.

The data matrix is often pretreated so that score values, loading values and residual matrix are obtained. The obtained score and loading plots allow an efficient interpretation of the whole data space. Residual plots contain noise. Figure IV.1 shows a typical PCA plot wherein the scores of the data are plotted in a 3D space with respect to the two main principal components and the score value with respect to the loading vector.



(A) (B)

Figure IV.1 (A) PCA plot in a 3D space and
(B) Plot showing the of score value with respect to the first loading for three variables

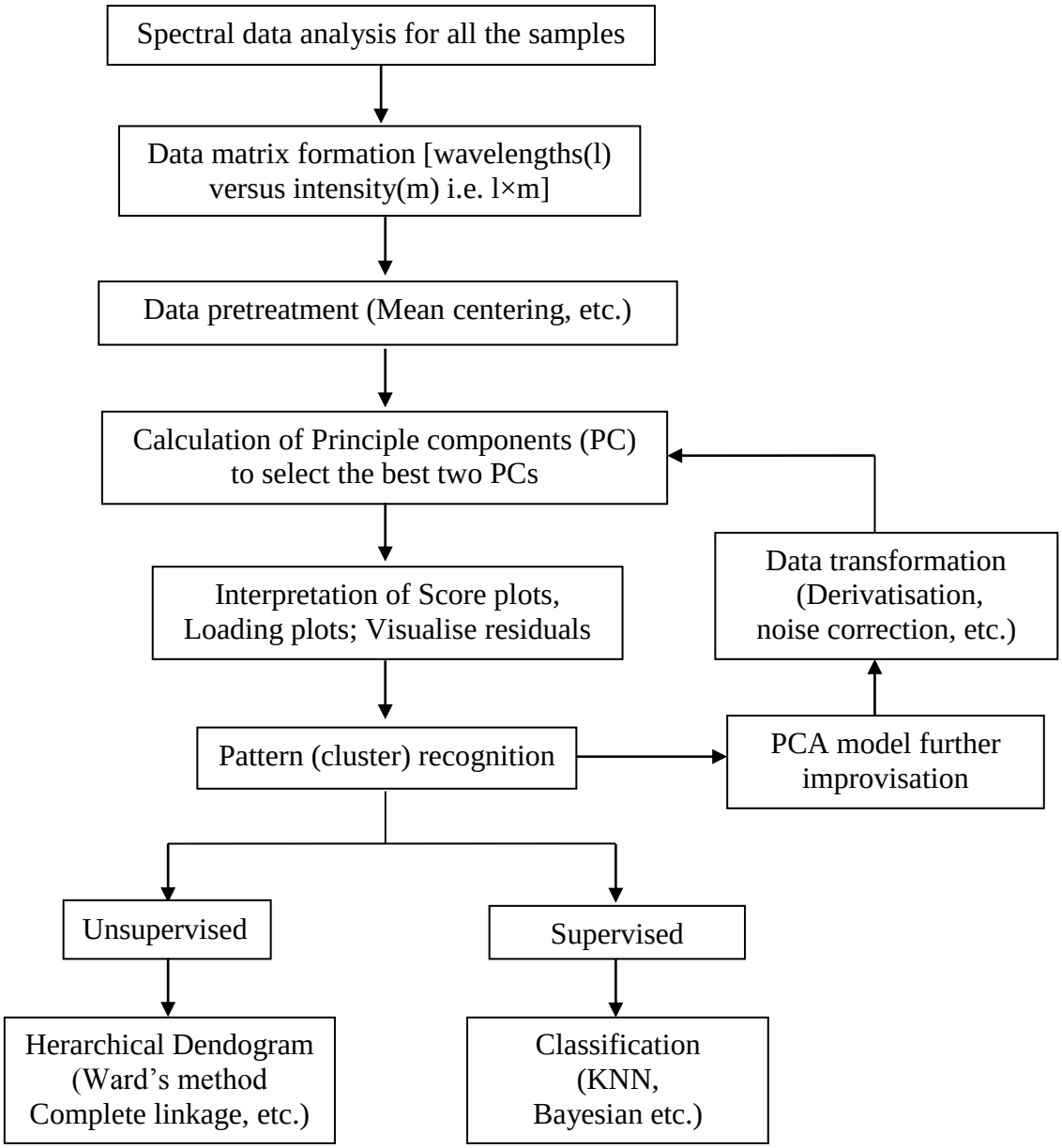
Score plots: Defines the Projection of data onto subspace. A typical score plot for two principle components (or latent variables) normally shows clusters (scattered/unscattered). Dense clusters depict samples with minimum variation and spread-out clusters depict larger variation. The outliers are normally totally different from clusters in their properties and may be useful for quality assessment. Sometimes clusters may be clear but in many situations there may be a gradient between them including gradual variability. Outliers may also be due to errors in sampling, data handling, number rounding or may be based on the existence of unknown classes of objects.

Loading plots: These plots define the contribution of variable in a particular model and show the relation between original variables and subspace dimensions. The outlier variable or that with least magnitude may be less impact on the model and can be removed in order to increase the accuracy of the model.

For getting better results and accuracy of the PCA model, the pretreatment and transformation of data is often carried out. The pretreatment methods include mean-scattering, weighting, etc. If the PCA model developed after pretreatment can further be improvised and resolved by various transformations such as Savitzky Golay derivatisation, Gap segment derivatisation, Norris gap derivatisation, Standard normal variate, etc.

Pattern recognition can be supervised or unsupervised. The supervised classification is non-herarchical and are based on Euclidean, Minkowski, Mahanobilis, etc. distances. Methods such Bayesian modeling and k-nearest neighbour are widely used for this cluster discrimination. In unsupervised situation, all the samples are measured and the data matrix is constructed including all data (no elimination of residuals). The clusters in unsupervised situation suggest the variability of samples and are classified using hierarchical cluster analysis (HCA) with various methods such as Ward's method, HCA single linkage, HCA complete linkage and HCA median linkage. In HCA the distance between the objects and cluster is computed which has significant impact on classification. The unsupervised HCA are based on Euclidean, squared Euclidean, Pearson correlation, Spearman's rank correlation, etc. distances.

The outline of the exploratory data analysis used in the present study is as follows:-



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DEVELOPMENT OF CHEMOMETRIC MODELS TO STUDY THE QUALITY OF AMOXICILLIN TRIHYDRATE AND POTASSIUM CLAVULANATE IN COMMERCIAL FORMULATIONS BY USING NIR, RAMAN AND FTIR TECHNIQUES

7.1. SELECTION OF FORMULATION

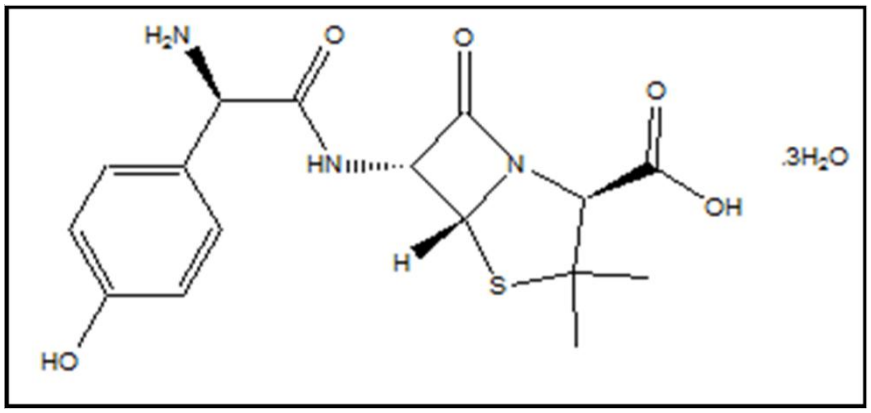
The Amoxicillin trihydrate (AMX) is one of the widely prescribed antibiotic medicine for cold and cough. It is routinely preferred by customers and easily available without the prescription of physicians. AMX is sometimes obtained from the pharmacy shops even without prescription. More than 30-40 brands in different formulations are available in Indian market. Being an antibiotic drug, a proper quality should be prevailed to ensure the patient safety and to maintain the pharma sector quality. Our survey has shown that the Food and Drug Laboratory, Vadodara (FDL) has reported the availability of some counterfeit AMX formulations in the market. Hence it was planned to develop chemometric models to assess the quality of various brands of AMX available in local market.

The study was further extended to the quality assessment of the combination of Amoxicillin trihdrate and Potassium clavulanate (PC) which is one of the well-known prescribed antibiotic combination in the market. A number of brands well recognized or local are available for this combination in the market. The AMX alone or in combination with PC is being widely prescribed by doctors and used by patients which justifies the availability of large number of brands for this particular drug and combination. Due to this reason the quality of the product may vary from each other and may not be upto- the-mark according to the ICH compliance. Hence the quality or variability among various brands was assessed with analytical techniques aided with chemometrics.

In routine practice NIR and Raman techniques are widely used for assessing the quality of drugs and their formulations. IR technique is not so oftenly used for this purpose; however IR is easily available and is often preferred for qualitative analysis. Hence alongwith NIR and Raman, the FTIR technique has also been explored to develop models for the quality assessment.

7.2. DRUG PROFILE

(1) Amoxicillin trihydrate (AMX) (1, 3)



a. Category : Antibiotic

b. Molecular formula : $C_{16}H_{25}N_3O_8S$

c. Molecular Weight : 419gm/mole

d. Nomenclature:(2S,5R,6R)-6-{[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl] amino}-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

e. Physicochemical Properties:

i. Description: White powder

i. Solubility: Freely soluble in methanol, acetonitrile

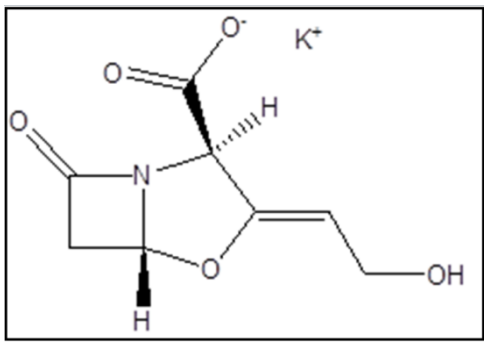
ii. log P: 0.75

iii. pK_a: 3.23, 7.43

iv. Melting Point: >200 ºC

f. Official Status: Official in IP, USP and BP.

(2) Potassium clavulanate (PC) (2,3)



g. Category : Antibiotic

h. Molecular formula : $C_8H_8KNO_5$

i. Molecular Weight : 237gm/mole

j. Nomenclature: potassium (Z,2R,5R)-3-(2-hydroxyethylidene)-7-oxo-4-oxa-1-aza-bicyclo[3.2.0]heptane-2-carboxylate

k. Physicochemical Properties:

i. Description: White powder

ii. Solubility: Freely soluble in methanol, acetonitrile

ii. log P: -1.5(clavulinic acid)

iii. pK_a: 3.32, -2.6

iv. Melting Point: >200ºC

l. Official Status: Official in IP, USP and BP.

The combined formulation of AMX and PC is official in IP, BP and USP.

7.3. LITERATURE REVIEW

Several reports are available using chemometric methods for detection of AMX individually or with other drugs, such as LC (4), TLC (5) and HPTLC (6). The studies for counterfeiting of such antibiotics have been carried out in various regions such as Middle East (7), Myanmar and Vietnam (8), Asia (9), Ghana (10) and Combodia (11, 12, 13) and various health reports (14, 15) are available in the literature. Literature reveals reports for counterfeits sale on online pharmacies in India (16). Khan et al (17) have reprted a detailed study of the spurious/falsely labelled/ falsified/counterfeit (SFFC) drugs in India from the year 2000-2013, wherein various such cases have been reported in regions of Delhi, Jaipur, Mumbai and Faridabad. Although a decline in substandardisation of drugs has been reported from the year 1995 to 2009 (18-20). The study of the quality of AMX and PC by IR, NIR and Raman techniques has not been reported yet.

7.4. EXPERIMENTAL

7.4.1. Instrumentation and Softwares

IR-Affinity Fourier Transform infrared (FTIR) spectrometer, Shimadzu, was used for FTIR analysis equipped with IR solution software.The samples were analysed by Miracle 10 single reflection attenuated total reflectance accessory.

The NIR analysis was carried out by Thermo Scientific NIR, micro-PHAZIR RX Analyzer equipped with Method Generator software version 4.

The Raman spectroscopy was performed on Sciaps Raman Spectrometer, Inspector 300 model and the data processing was carried out with NuSpec software. The raman signals were detected using high resolution CCD detector.

For chemometrics, Unscramber software 10.3 version was used for the data processing and analysis. All recorded spectra were background corrected. PCA and CA techniques were applied to the spectral data. The goal of this step was to differentiate the original formulational spectra from the counterfeit and placebo samples.

7.4.2. Materials and reagents

The AMX bulk drug was kindly gifted by Kaptab pharmaceuticals ltd, Vadodara. The excipients used for preparation of substandard laboratory samples as well as placebo samples i.e. sodium starch glycolate, microcrystalline cellulose, talc, hypromellose, titanium dioxide, colloidal silica, magnesium stearate, starch, cross povidone, aspartame, saccharin, mannitol, lactose, dibasic calcium phosphate and cross carmellose sodium were of SDFine Chemicals.

7.4.3. Sample collection and preparation

The total samples to be analysed comprised of various commercial formulation (FM) samples, collected from the local pharmacy stores including the laboratory prepared standard samples of AMX and AMX-PC and the laboratory prepared counterfeit (CF) samples and placebo (PL) samples.

The capsule as well as tablet formulations, which were to be analysed, were powdered, filled in a polythene bag. 100 mg of powder mixture was taken, spreaded over the previously marked area of $1cm^2$ on the polythene bag and were analysed by Raman and NIR. For FTIR analysis the powdered samples were directly put under the ATR probe and analysed. The powdered samples of counterfeit and placebo samples were analysed in a similar way.

7.4.4. Spectral data

All samples (counterfeit, original and placebo) for each drug were first measured by the FTIR. The scan covered the range (4000-600cm^{-1}) nm (about 2 nm increments). The spectrum of each sample was an average of three scans. A matrix of 55×1763 (55 samples and 1763 wavelengths) was formed in case of AMX study and 36×1763 (36 samples and 1763 wavelengths) in case of AMX-PC study, which was subjected to chemometric modelling.

The samples were then measured by NIR. The scan covered the range (700-2500cm^{-1}) (about 8 nm increments). The spectrum of each sample was an average of five scans. A matrix of was 55×100 (55 samples and 100 wavelengths)formed in case of AMX study and 36×100 (36 samples and 100 wavelengths) in case of AMX-PC study, which was subjected to chemometric modelling.

The samples were analysed by Raman spectrometer using a laser of 785 nm wavelength for excitation. The raman signals scanned over a range of 500-2500 cm^{-1} raman shift with 30 seconds of intergration time. A matrix of was 55×2301 (55 samples and 2301 wavelengths) formed in case of AMX study and 36×2301 (36 samples and 2301 wavelengths) in case of AMX-PC study, which subjected to chemometric modelling.

7.5. CONSTRUCTION OF MODELS

Every drug formulation has a unique spectral fingerprint in the NIR, Raman and IR spectra, that identifies the brand of the drug. Incorrect formulations containing foreign or substitute ingredients can put the patient's life under risk. Taking this basic concept to compare the entire spectra, the PCA, one of the chemometric techniques, was used to detect fine differences in the spectra.

The PCA works on the principle that it finds the directions (or vector) in the space of data along which the dispersion (or variance) of the data is maximum. The directions or vectors are called principle components (PCs). The PCs are calculated in such a way that the first PC carries majority or maximum information which is statistically termed as "Explained variance". The second PC is thus calculated and carries maximum information or variance that has not been included by the first PC.

Similarly the PCs are further calculated (PC3, PC4 and so on) till all the information of the data has been accounted. The two PCs which explain most of the variance are selected for buiding the PCA model. In predicting PCA score plots, samples that form a cluster are believed to belong to the same origin. Each one of the groups of samples represents certain similarity and for the present study it represented similar form of the samples (counterfeit, original and placebo) (21).

For further investigation of the samples, hierarchical cluster analysis (HCA) was also used. The Ward error sum of squares hierarchical clustering method was used for the study. The clustering by Ward's method is based on minimum variance. Ward's minimum variance criterion minimizes the total within-cluster variance. At each step the pair of clusters with minimum cluster distance are merged. To implement this method, at each step we find the pair of clusters that leads to minimum increase in total within-cluster variance after merging. This increase is a weighted squared distance between the cluster centers. At the initial step, all clusters are singletons (clusters containing a single point) and subsequently form large clusters as we move on to the upper side of dendrogram (22).

The PCA was also performed by applying the Savitzky-Golay derivative method. The first derivative algorithm was applied to the normal zero order spectra by using SGolay algorithm and then PCA and HCA were performed. Savitzky-Golay is used for calculating smoothing and differentiation of data by least-squares technique. The Savitzky-Golay approach has been widely used because it produces a significant improvement in the lengthy least-squares calculation by making a simple and equivalent, convolution. In this approach the least-squares value of a given point is calculated as a weighted combination of itself and m points on either side of it. This corresponds to performing a moving (2m + 1 point) least-squares fit across the data (23, 24).

7.6. METHOD 7A. STUDY OF THE QUALITY OF AMX BRANDS

7.6.1. MARKETED FORMULATIONS AND LABORATORY SAMPLE PREPARATION

A wide range of formulations is available but only tablets and capsules were selected for the study. Various commercial formulations i.e. dispersible tablets and capsule of AMX were bought from various pharmacy stores and were used for analysis. The tablets and capsules with label claim of 125 mg, 250 mg or 500 mg AMX were analysed. All the tablets were uncoated tablets. Total 35 samples were used in the study of AMX The various brands undertaken for study are listed in Table 7.1.

Table 7.1. List of the marketed and laboratory formulations of AMX (AMX FM)

Company Name	Formulation	Quantity of AMX per formulation	Sample Code
Alkem	Capsule	250	AC1
Abott (megamox)	Capsule	250	AC2
Abott (ronemox)	Capsule	250	AC3
Aarsh	Capsule	500	AC4
Biochem	Capsule	500	AC5
Cadilla	Capsule	250	AC6
Cipla	Capsule	250	AC7
Laborate	Capsule	250	AC8
Manish	Capsule	250	AC9
Pelican	Capsule	500	AC10
Ranbaxy	Capsule	250	AC11
Smart	Capsule	250	AC12
Wyeth	Capsule	250	AC13
ZydusCadilla	Capsule	250	AC14
Lupin	Capsule	500	AC15
AIOC	Tablet	250	AT1
Lab prepared tablet	Tablet	250	*AT2
Abott (Ronemox)	Tablet	250	AT3

Abott (Megamox)	Tablet	250	AT4
Blue Cross (kidtab)	Tablet	125	AT5
Blue Cross	Tablet	250	AT6
Exotic	Tablet	250	AT7
Cipla (kidtab)	Tablet	125	AT8
Dey's	Tablet	250	AT9
Exotic	Tablet	125	AT10
Invision	Tablet	250	AT11
Intas	Tablet	125	AT12
Kaptab (kidtab)	Tablet	125	AT13
Kaptab	Tablet	250	AT14
Ranbaxy	Tablet	250	AT15
Ranbaxy (kidtab)	Tablet	125	AT16
Sandoz	Tablet	125	AT17
Veritaz	Tablet	250	AT18
Centurion	Tablet	250	AT19
ZydusCadilla	Tablet	125	AT20

Underlined samples comprise test set; *Laboratory prepared

Three type of laboratory samples were prepared in the lab (25, 26): (I) Standard tablets of AMX were prepared in laboratory as per the formula mentioned in Table 7.2, (II) Samples with no AMX i.e. Placebo (PL) samples (containing only excipients) as mentioned in Table 7.3. Ten placebo tablet mixtures were prepared in the laboratory and (III) Substandard samples (or counterfeit samples) (CF samples) were prepared as per Table 7.4, having varied (low or high) concentration of AMX combined with other drugs such as paracetamol, aspirin, diclofenac, etc.

The addition of other drugs as additive to prepare spurious drugs was undertaken to get a wider variety for the counterfeit samples. Some common drugs such as aspirin, paracetamol, diclofenac, ibuprofen, etc. were selected as they are easily available and cheaper than the AMX. Other antibiotics drugs such as azithromycin and cefpodoxime proxetil were selected to see the effect of other antibiotics (on the results of chemometric modes). Other drugs such as ramipril, simvastatin, atenolol, phenylephrine were randomly selected (blind selection). The variety in the

counterfeits was selected to have a wider scope of counterfeits and to prepare the most reobust chemometric models. Ten counterfeit tablet mixtures were prepared as mentioned in Table 7.4 in the laboratory.

Table 7.2 Composition of Laboratory prepared AMX tablets (25,26)

Ingredients	AMX Tablet
Amoxicillin trihydrate	286.98 mg
Colloidal silica	5 mg
Cross carmellose sodium	20 mg
Magnesium stearate	7.5 mg
Microcrystalline cellulose	170.52 mg
Aspartame	10 mg
Total weight	500 mg

Table 7.3. Composition of the placebo (PL) samples

	Amount in mg									
Ingredients	PL1	PL2	PL3	PL4	PL5	PL6	PL7	PL8	PL9	PL10
Cross Carmellose Sodium	25	--	35	--	50	--	60	--	75	--
Sodium Starch Glycollate	--	25	--	--	--	75	--	--	--	--
Starch	75	--	50	--	25	--	30	--	60	--
Magnesium stearate	--	--	--	37.5	--	--	--	--	--	25
Cross Povidone	--	--	--	--	--	--	--	50	--	25
Aspartame	50	--	60	--	75	--	85	--	50	--
Sacharrin	--	75	--	50	--	25	--	35	--	75
Micro crystalline cellulose	--	25	--	37.5	--	50	--	5	--	15
Talc	--	--	25	--	12	--	25	--	--	--
Colloidal silica	5	--	--	--	--	--	--	--	20	--
Mannitol	345	--	330	--	338	--	--	--	--	--
Lactose	--	375	--	375	--	--	300	--	295	--
Dibasic calcium phosphate	--	--	--	--	--	350	--	410	--	360
Total weight	500 mg									

Underlined samples comprise test set

Table 7.4. Composition of the counterfeit (CF) samples

Ingredients	Amount in mg									
	CF1A	CF2A	CF3A	CF4A	CF5A	CF6A	CF7A	CF8A	CF9A	CF10A
AMX	50	75	100	150	80	20	140	125	90	100
Cross Carmellose Sodium	25	--	35	--	50	--	60	--	75	0
Sodium Starch Glycollate	--	25	--	--	--	75	--	--	--	--
Starch	75	--	50	--	25	--	30	--	55	--
Magnesium stearate	--	--	--	37.5	--	--	--	--	--	28
Cross Povidone	--	--	-	--	--	--	--	50	--	25
Aspartame	50	--	60	--	75	--	85	--	50	--
Sacharrin	--	75	--	50	--	25	--	35	--	75
Micro crystalline cellulose	--	25	--	37.5	--	50	--	5	--	12
Talc	--	--	25	--	10	--	--	--	20	--
Colloidal silica	5	--	--	--	--	--	25	--	--	--
Mannitol	195	--	170	--	130	--	--	--	--	--
Lactose	--	175	--	150	--	--	90	--	125	--
Dibasic calcium phosphate	--	--	--	--	--	190	--	230	--	170
Other additive	100	125	60	75	130	140	70	55	85	90
Total weight	500 mg									

Underlined samples comprise test set

The other additives used were CF1A: Aspirin; CF2A: Paracetamol; CF3A: Diclofenac; CF4A: Atenolol; CF5A: Ramipril; CF6A: Azithromycin; CF7A: Phenylephrine; CF8A: Ranitidine; CF9A: Ibuprofen; CF10A: Cefpodoxime proxetil.

A blinded test (or validation) set was built up in order to check the model accuracy. The test set comprised of 5 samples viz: AC3, AT6, AT12, PL5, CF5A

7.6.2. RESULT AND DISCUSSION

7.6.2.1. NIR- chemometric models

7.6.2.1.1. NIR (zero order) PCA model

The Figures 7.1 shows the overlay of the zero order spectra for all the AMX samples including the FM, CF and PL. Figure 7.2 shows the PCA plot for the analysis of NIR spectra (zero order) of AMX samples. The clusters of the PCA plot are described in Table 7.5.

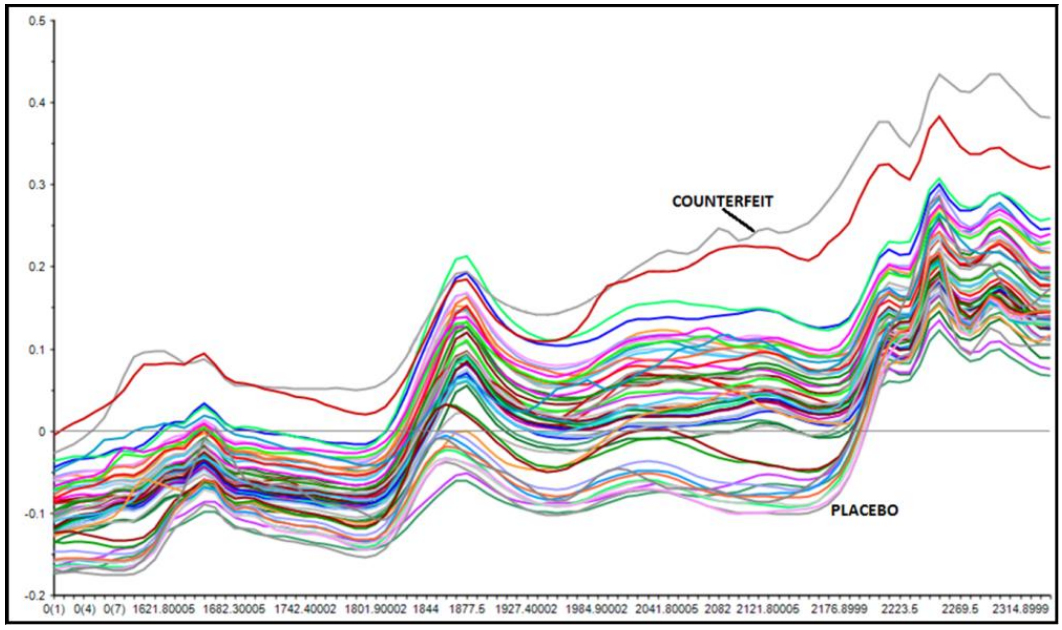


Figure 7.1 Overlay of zero order NIR spectra of the AMX FM samples, CF samples and PL samples

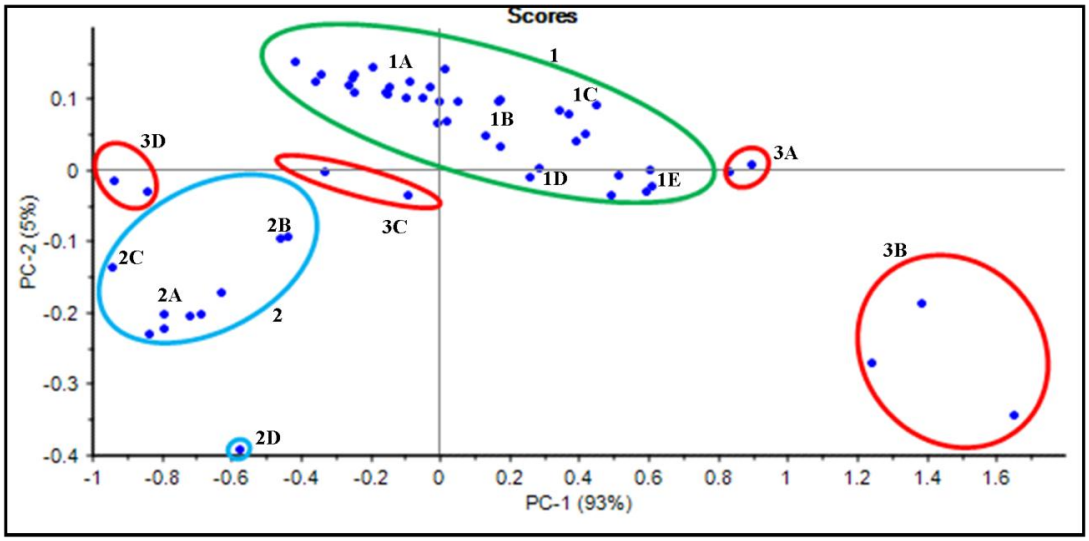


Figure 7.2 PCA plot for the zero order NIR spectra of AMX FM samples, CF samples and PL samples

Table 7.5 Description of clusters formed as per PCA plot in Figure 7.4

Sample	Cluster No.	Samples in the cluster
FORMULATION	1A	AC2, AC3, AC5 to15, AT6, AT9, AT11, AT12, AT14, AT17, AT18
	1B	*AT2, AT5, AT8,
	1C	AC1, AC4, AT4, AT13, AT15
	1D	AT3, AT7
	1E	AT1, AT10, AT16, AT19, AT20
PLACEBO	2A	PL1
	2B	PL6, PL7
	2C	PL2, PL3, PL4, PL5, PL8, PL9
	2D	PL10
COUNTERFEIT	3A	CF2A, CF10A
	3B	CF1A, CF6A, CF7A, CF9A
	3C	CF5A, CF8A
	3D	CF3A, CF4A

PCA of the data matrix showed 7 PCs as given in Table 7.6. The percentage explained variance in the table is given in terms of cumulative explained variance (PC1 explained variance is 92.33%, PC2 explained variance is 97.47-92.33=5.14% and so on). The PC1 and PC2 explain maximum variance and hence are used for building the PCA model.

Table 7.6 Explained variance of the PCs

	Percentage Explained variance						
PCs	1	2	3	4	5	6	7
CAL	92.33	97.47	98.88	99.3	99.59	99.72	99.81
VAL	91.14	96.79	98.38	98.83	99.18	99.32	99.40

PCs= principle components, CAL=calibration; VAL=validation

Seven significant clusters were found i.e. one cluster for AMX FM samples (1) (green colour), 4 subclusters for placebo (2A-D) (blue colour) and four clusters for CF samples (3A-D) (red colour). The green cluster that represented the AMX FM samples showed gradient effect due to variability of different brands and within this cluster, it can be easilY concluded that quality of formulations comprising 1A group is quite comparable and variability of 1A and 1E group within the cluster is quite distinct. The CF samples formed four different clusters; it was expected as all the CF

samples were quite different from each other in their chemical composition. The test set samples in the PCA plots were found within the training set sample clusters 1A. 1B cluster comprised of the lab formulation which was present exactly at the centre of the cluster 1.

7.6.2.1.2. NIR (SGolay derivative) PCA model

Another model was prepared after the spectra were treated with SGolay first derivative calculation. The Figures 7.3 and 7.4 show the overlay of the derivative spectra for all the AMX samples including the FM, CF and PL. The PCA model was developed for the SGolay derivative data as shown in Figure 7.5, wherin the derivative spectra of samples were taken and the PCA plot was constructed. The details of clusters have been shown in Table 7.7.

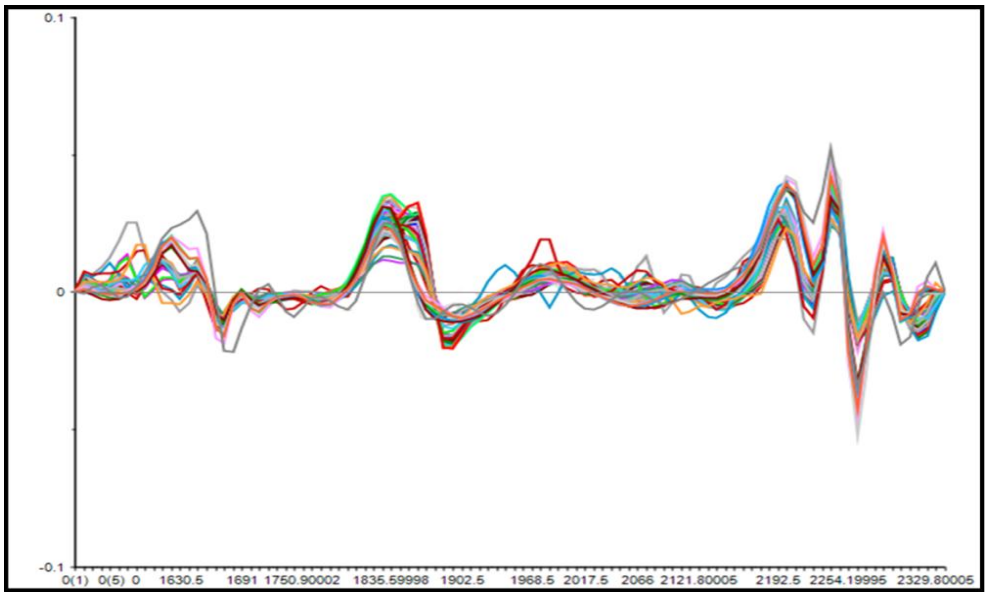


Figure 7.3 Overlay of the first derivative NIR spectra of AMX FM samples, CF samples and PL samples

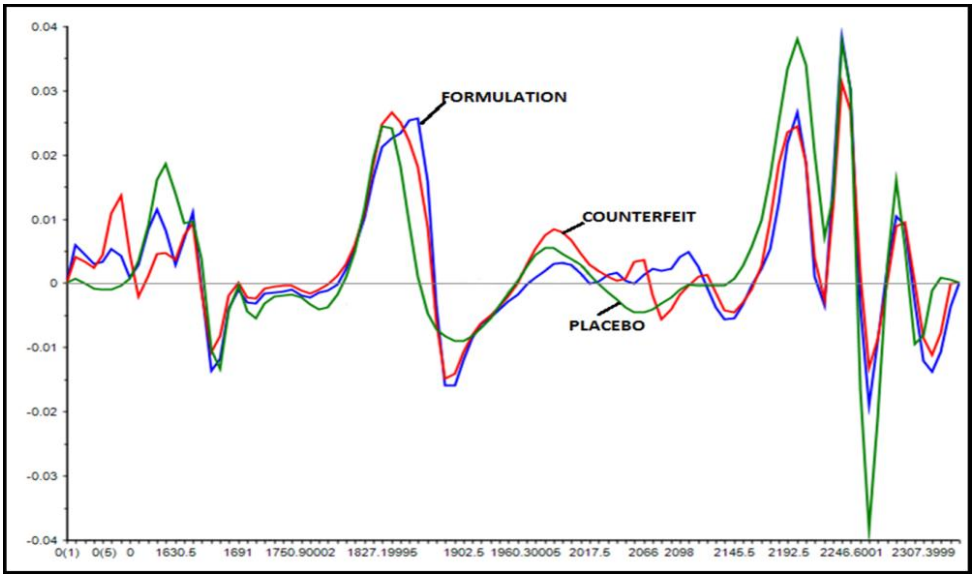


Figure 7.4 Overlay of the first derivative NIR spectra of AMX FM sample, CF sample and PL sample (for easy differentiation)

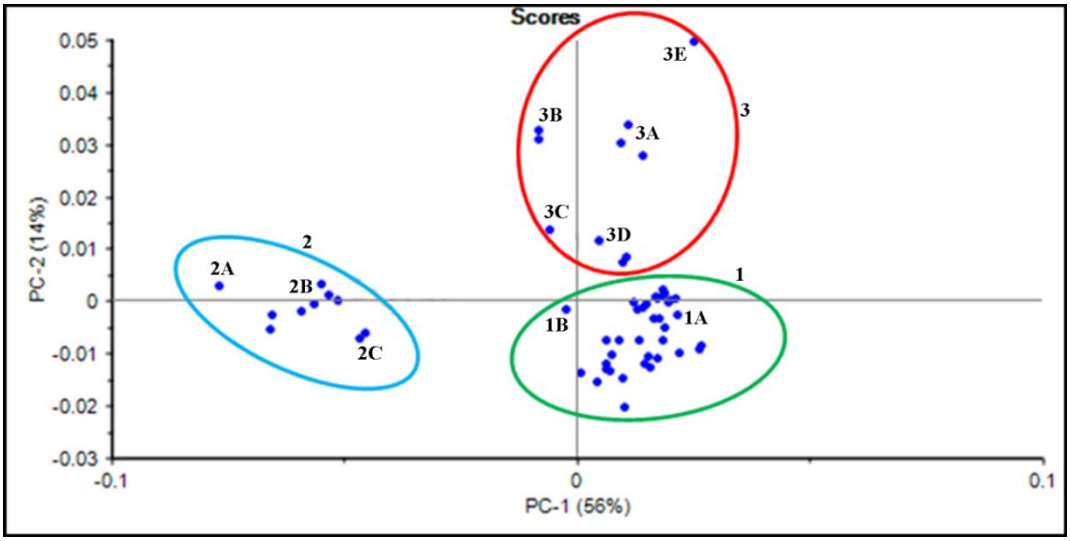


Figure 7.5 PCA plot for the first derivative NIR spectra of AMX FM samples, CF samples and PL samples

Table 7.7 Description of clusters formed as per PCA plot in Figure 7.5

Sample	Cluster No.	Samples in the cluster
FORMULATION	1A	AC1, AC2, AC3, AC4 to AC15, AT1 to AT5, AT6, AT7 to AT11, AT12, AT13, AT15 to AT20
	1B	AT14
PLACEBO	2A	PL10
	2B	PL1, PL2, PL3, PL4, PL5, PL8, PL9
	2C	PL6, PL7
COUNTERFEIT	3A	CF2A, CF9A, CF7A
	3B	CF3A, CF4A,
	3C	CF8A
	3D	CF6A, CF5A, CF10A
	3E	CF1A

The percentage explained variance of the 7 PCs calculated for the model is given in Table 7.8. The PC1 and PC2 showing maximum values were used to construct the PCA plot.

Table 7.8 Explained variance of the PCs

	Percentage Explained variance						
PC	1	2	3	4	5	6	7
CAL	56.42	70.01	78.84	84.23	88.62	91.45	93.7
VAL	54.11	63.49	71.83	73.19	76.48	78.24	79.44

The PCA plot showed three distinct clusters of AMX samples viz. (1) one for AMX FM samples (green colour), (2) three sub-clusters for PL samples (blue colour) and (3) five sub-clusters for CF samples (red colour) which has been described in Table 7.5. Cluster 1A in the plot was quite densely populated and comprised of almost all FM samples except sample AT14. The formation of dense plot reduced the variability of different brands bringing them to closer affinity. The test set samples in the PCA plots were also found within the cluster 1A. Cluster 2B contained maximum PL samples including the test ones. The five CF subcluster though were little scattered but quite differentiating from the FM samples. The cluster formation and density of the clusters was quite better than that obtained for the zero order NIR PCA model. This was indeed a good plot to identify outliers i.e. for SFFC drugs.

7.6.2.1.3. NIR HCA model

The hierarchical cluster analysis shown in Figure 7.6 showed distinct classification for the three groups. The clusters for PCA and cluster analysis could successfully and clearly differentiate the AMX FM samples from the PL and CF ones. Four distinct clusters were found i.e. one for placebo, one for CF (except CF5A and CF10A which were closer to AMX FM samples), one cluster for AMX capsule FM samples and one for tablet FM samples. HCA describes the similarities and variabilities among various samples e.g. among commercial formulations, going from AC15 to AT14. CF10A and CF5A are similar to AT14.

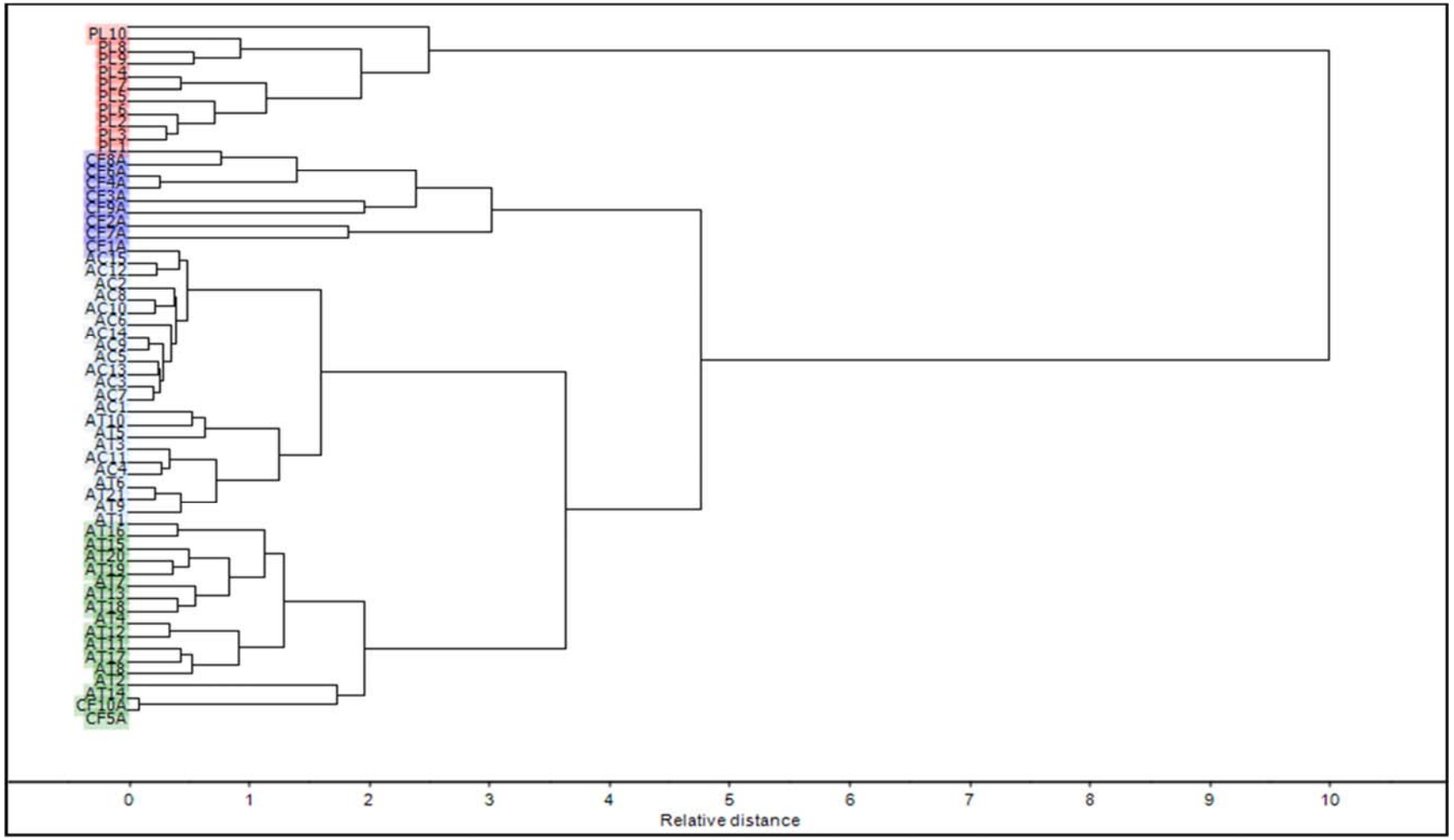


Figure 7.6 Cluster analysis plot for first derivative NIR spectra of AMX FM samples, CF samples and PL samples

7.6.2.2. Raman- chemometric models

7.6.2.2.1. Raman PCA model

Figure 7.7 shows the overlay of the Raman spectra of all AMX samples including CF and PL samples. Figure 7.8 shows the PCA plot for the analysis of Raman spectra of AMX samples (including placebo and counterfeit). The clusters have been described in Table 7.9.

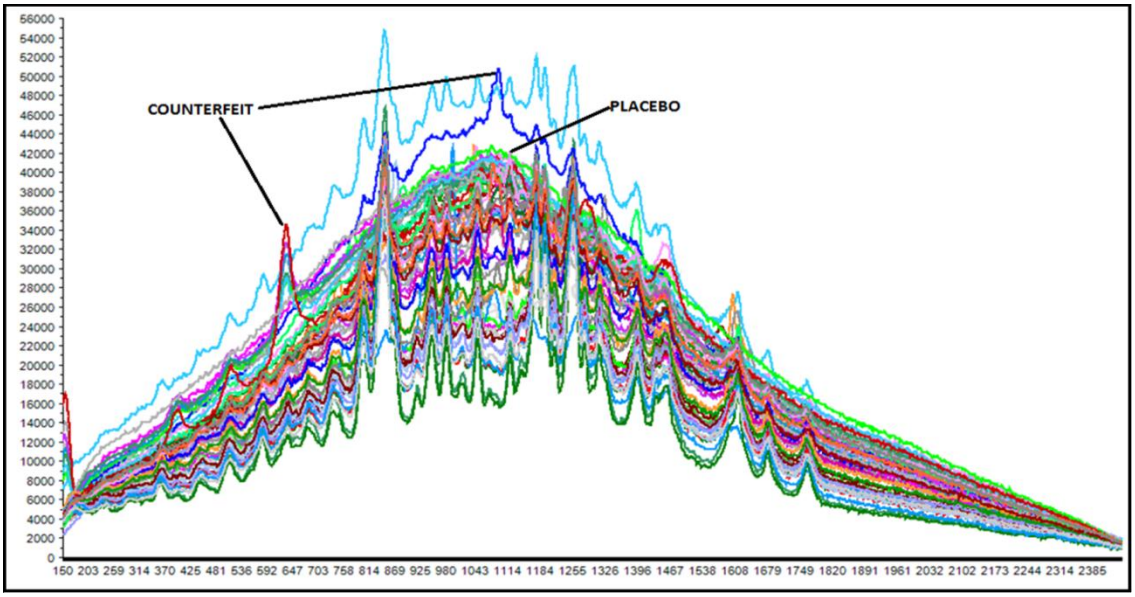


Figure 7.7 Overlay spectra of the zero order Raman spectra of AMX FM samples, CF samples and PL samples

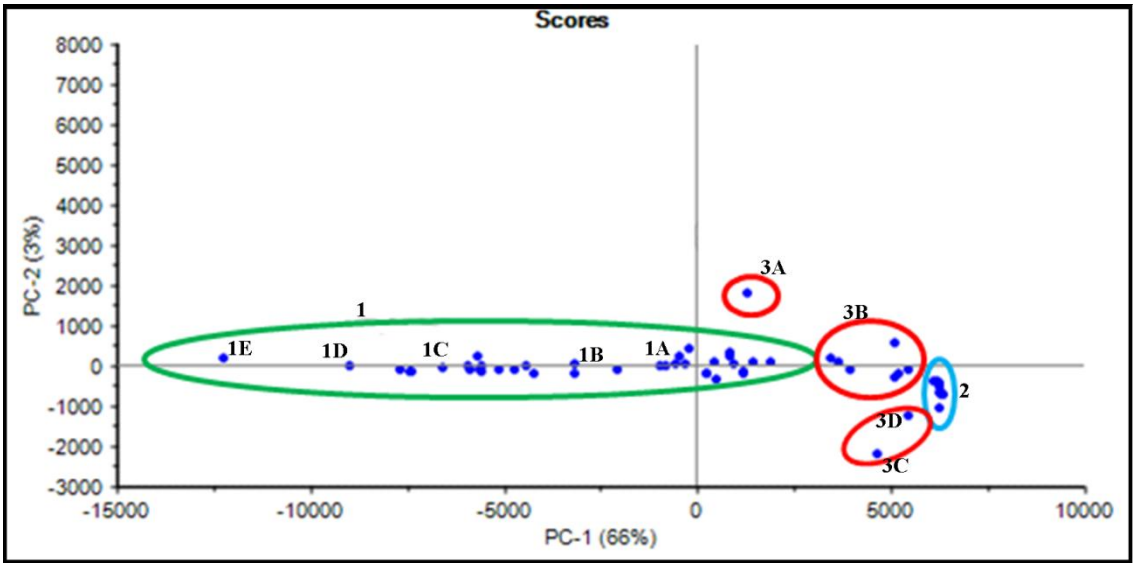


Figure 7.8 PCA plot for the zero order Raman spectra of AMX FM samples, CF samples and PL samples

Table 7.9 Description of clusters formed as per PCA plot in Figure 7.8

Sample	Cluster No.	Samples in the cluster
FORMULATION	1A	AT1, *AT2, AT4, AT5, AT6 to AT8, AT10, AT11, AT12, AT13 to AT20
	1B	AT6, AT14, AT18
	1C	AC1, AC2, AC3, AC4, AC5, AC7, AC9-15, AT3, AT9,
	Other	1D-AC6; 1E-AC8
PLACEBO	2	PL1 to PL4, PL5, PL6 to PL10
COUNTERFEIT	3A	CF3A
	3B	CF1A, CF4A, CF5A, CF6A to CF9A,
	3C	CF2A
	3D	CF10A

The percentage explained variance of the 7 PCs calculated for the model is given in Table 7.10. The PC1 and PC2 showing maximum values were used to construct the PCA plot.

Table 7.10 Explained variance of the PCs

	Percentage Explained variance						
PC	1	2	3	4	5	6	7
CAL	66.28	69.68	72.79	75.57	77.46	79.12	80.52
VAL	63.89	64.06	64.10	65.81	66.26	66.46	66.88

Raman spectra is affected by a number of sample parameters e.g. sample particle size, uniformity of layer, etc. This may lead to a lot of noise in the data. Hence the PCA plot was constructed after performing the baseline (or noise) correction of the spectral data. The PCA plot showed five significant clusters were found as described in Table 7.9 viz: (1) one cluster for AMX FM samples (5 subclusters 1A-E) (green colour), (2) one cluster for PL (blue colour) and four sub-clusters for counterfeit (3A, 3B, 3C, 3D) (red colour) and. The model shows five different clusters and specifically could differentiate the AMX FM samples from the CF and PL samples. The sample clusters of AMX FM i.e. cluster 1A, 1B which are closer to CF sample cluster 3A, were of tablet. The cluster 1C formed a gradient with a mixture of tablets and capsule samples i.e. a gradient from tablet to capsule FM from right to left. The sub-clusters 1D and 1E were quite far from cluster 1A and CF clusters and exclusively comprised of capsule FM samples. This showed the variability of the capsule FM samples from the tablet FM samples. The test set FM samples were present in major clusters: 1A and 1C, and test CF samples in the clusters 3B and 3C. The lab FM sample *AT2 was present in main cluster 1A.

7.6.2.2.2. Raman HCA model

The hierarchical cluster analysis plot is shown in Figure 7.9 showed distinct classification for the three groups. Three major clusters were formed viz. one for capsule samples, one for tablet samples and one for mixture of CF and PL samples. The HCA classification of clearly differentiates the groups.

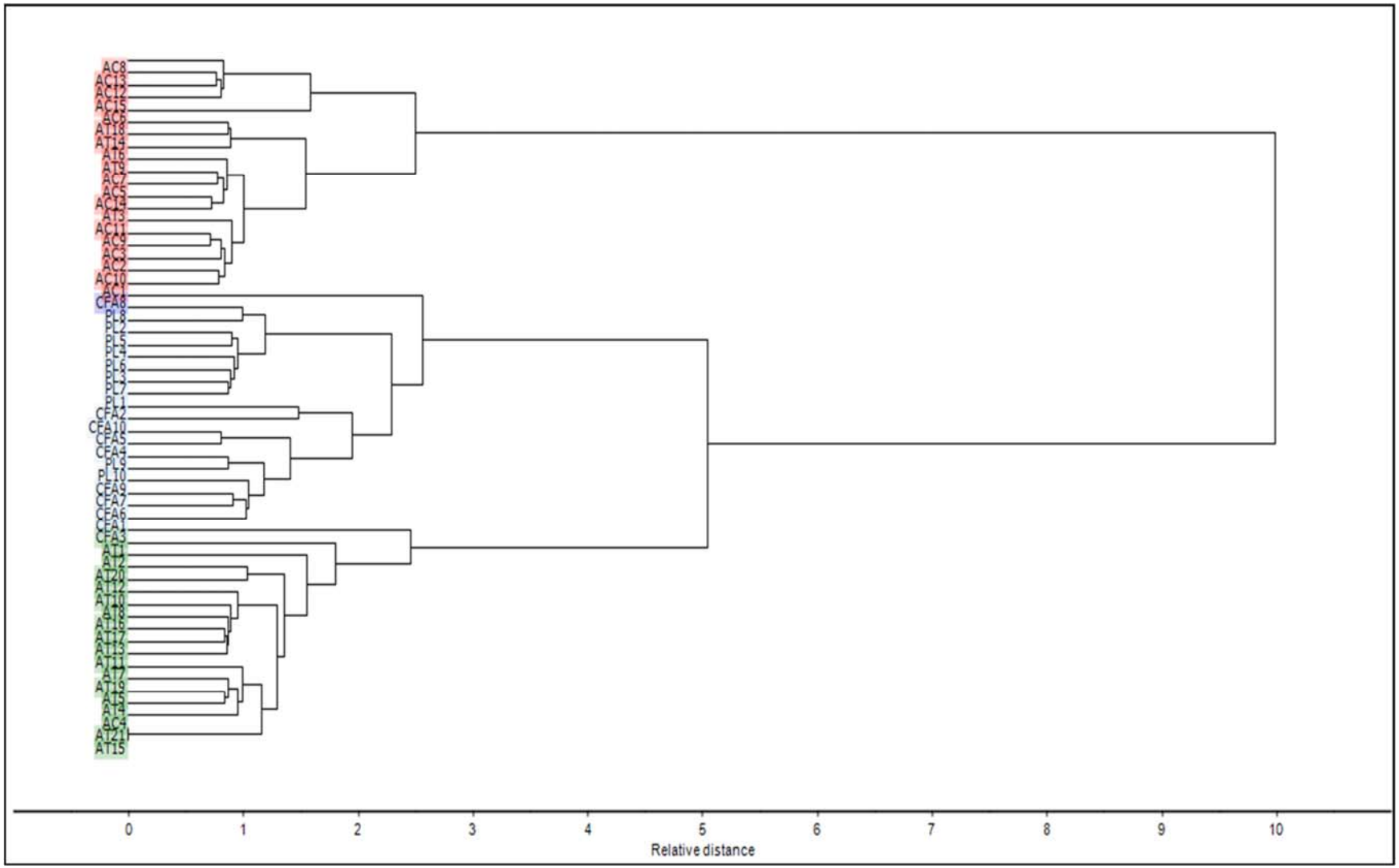


Figure 7.9 Cluster analysis plot for the Raman spectra of AMX FM samples, CF samples and PL samples

7.6.2.3. IR- chemometric models

7.6.2.3.1. IR (zero order) PCA model

The Figure 7.10 shows the overlay of all the IR spectra of AMX samples including FM, CL and PL. The PCA plot for the analysis of AMX samples are shown in Figure 7.11.

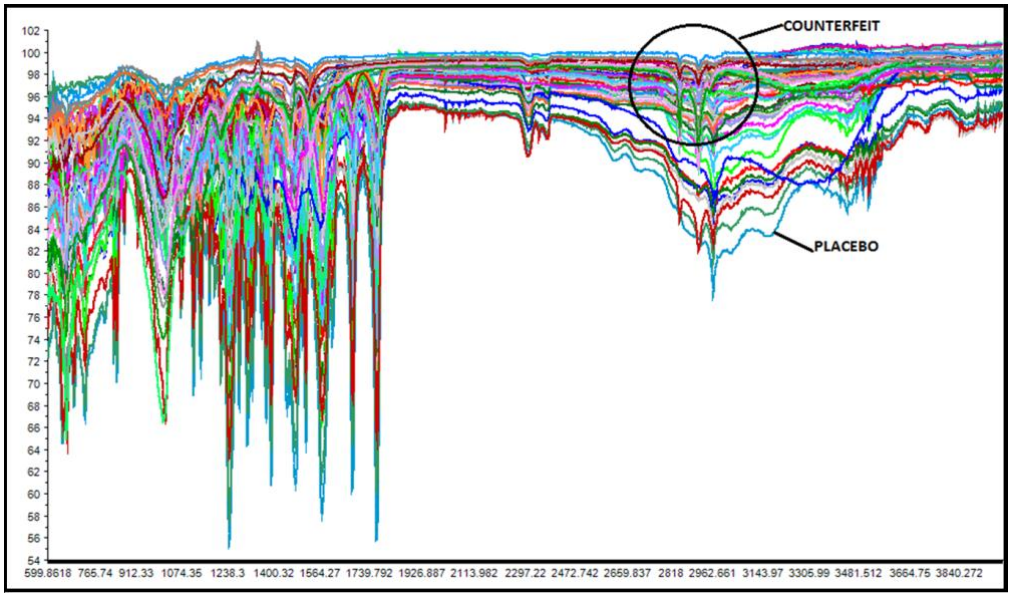


Figure 7.10 Overlay of zero order IR spectra of AMX FM samples, CF samples and PL samples

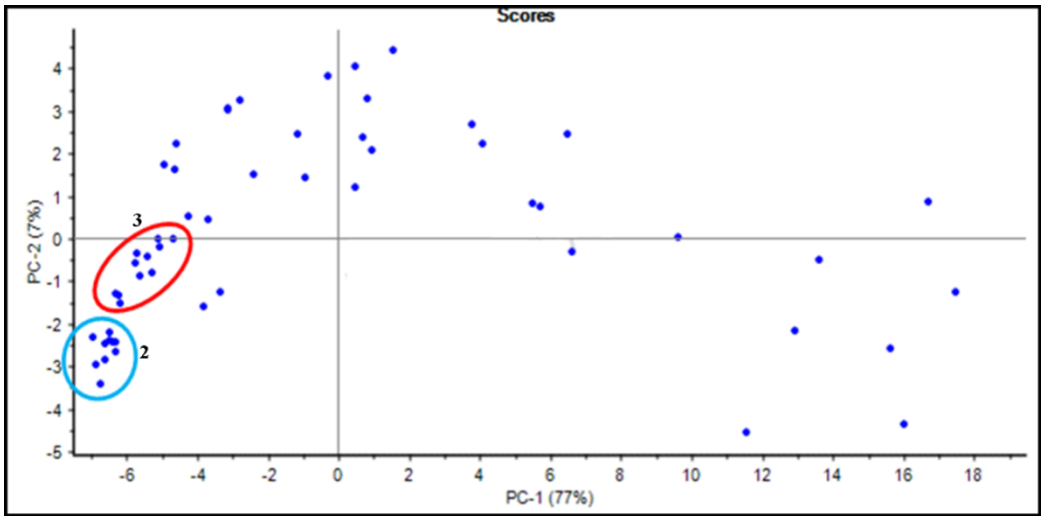


Figure 7.11 PCA plot for the zero order IR spectra of AMX FM samples, CF samples and PL samples

The PCA plot for zero order IR spectra was very scattered except that for the clusters of PL and CF samples which were found to be distinct. Hence no significant conclusion could be

reached. Moreover large number of wavelengths and more number of peaks so noise is higher. This makes difficult to extract meaningful information by simple PCA or HCA models.

7.6.2.3.2. IR (SGolay derivative) PCA model

Another IR PCA model was developed wherin the SGolay first derivative IR spectra were calculated. Figure 7.12 shows overlay of the derivative spectra of all the samples. The PCA plot of the derivatives of the IR spectra of AMX samples is shown in Figure 7.13. The different clusters are described in detail in Table 7.11.

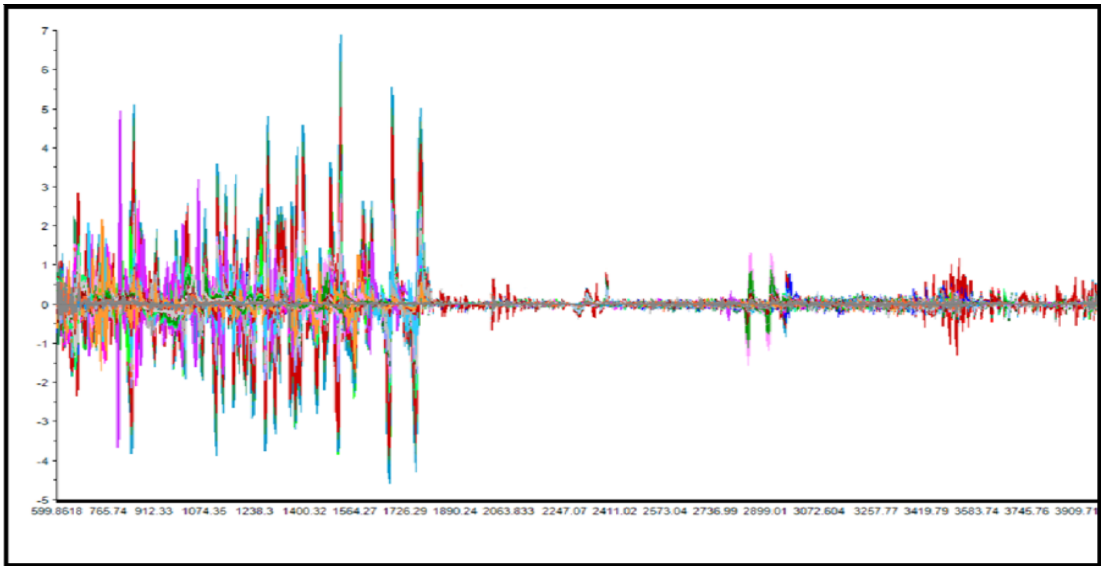


Figure 7.12 Overlay of the first derivative IR spectra of AMX FM samples, CF samples and PL samples

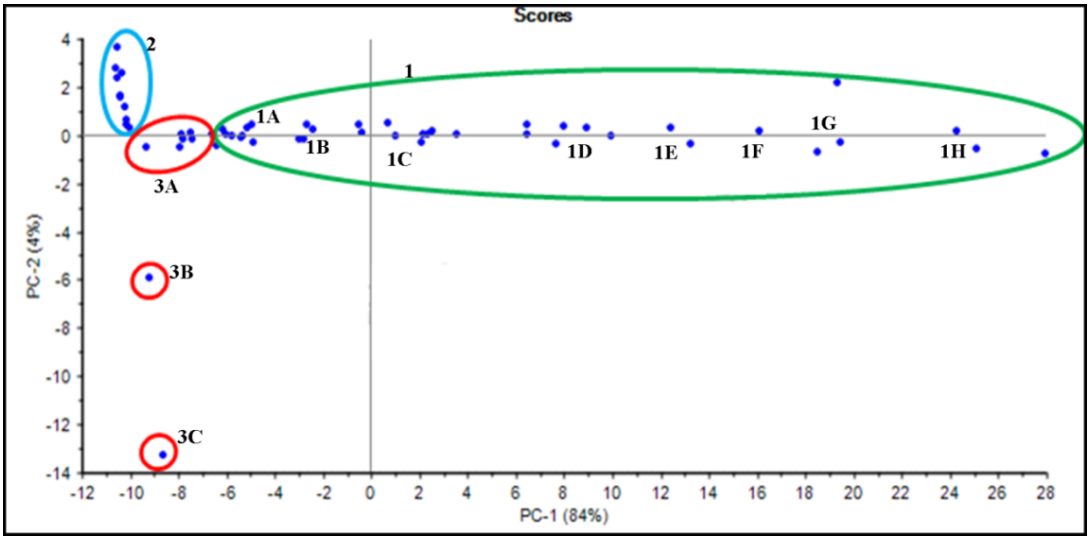


Figure 7.13 PCA plot for the first derivative IR spectra of AMX FM samples, CF samples and PL samples

Table 7.11 Description of clusters formed as per PCA plot in Figure 7.13

Sample	Cluster No.	Samples in the cluster
FORMULATION	1A	*AT2, AT4, AT8, AT9, AT12, AT13, AT16 to AT18,
	1B	AT1, AT8, AT19, AT20
	1C	AT3, AT5, AT7, AT10, AT11, AT14, AT15, AC3, AC8
	1D	AT4, AT6, AC5, AC7, AC11, AC15
	Other	1E-AC1, AC13; 1F-AC2; 1G-AC4, AC5, AC14; 1H- AC9, AC10, AC12
PLACEBO	2	PL1 to PL10
COUNTERFEIT	3A	CF1A, CF2A, CF4A, CF5A, CF8A, CF9A
	3B	CF10A
	3C	CF3A
	OTHER	CF6A, CF7A: In between (boundary of) cluster 1 and 2

The percentage explained variance of the 7 PCs calculated for the model is given in Table 7.12. The PC1 and PC2 showing maximum values were used to construct the PCA plot.

Table 7.12 Explained variance of the PCs

Percentage Explained variance							
PC	1	2	3	4	5	6	7
CAL	84.15	87.78	90.86	92.36	93.46	94.39	95.13
VAL	82.74	83.30	86.52	87.60	88.45	89.11	91.14

The PCA plot gave five main clusters for AMX samples: (1) one for FM samples (8 sub-clusters 1A-1H) (green colour), (2) one for PL samples (blue colour) and (3) three sub-clusters for CF samples (3A-3C) (red colour). The cluster 1A which was quite close to the CF cluster comprised of majority of tablet samples including the lab prepared AT2 sample, which may be due to similarity in the IR active excipients. The cluster 1 showed a wide gradient, even wider than Raman PCA plot, which comprised of total 8 subclusters. The gradient ranged from tablet to capsule samples from left to right direction showing the variabilities of these formulations. The plot showed clusters which could differentiate the FM samples from the CF and PL samples though the FM (cluster 1A) and CF samples were very close to each other. Few CF samples were also found on the boundary of cluster number 1 and 3A. Majority test FM samples plots were found in cluster 1C. Here also placebo comprised of a dense single cluster just like Raman-PCA model.

7.6.2.3.3. IR HCA model

The hierarchical cluster analysis plots showed different groups for AMX capsules, AMX tablets, mixture of counterfeit and AMX tablets, CF and PL samples. The mixture of clusters could be correlated with the gradient formed in the PCA plot hence the classification was not that clear as Raman-HCA and NIR-HCA dendrograms. The dendrogram is shown in Figure 7.14.

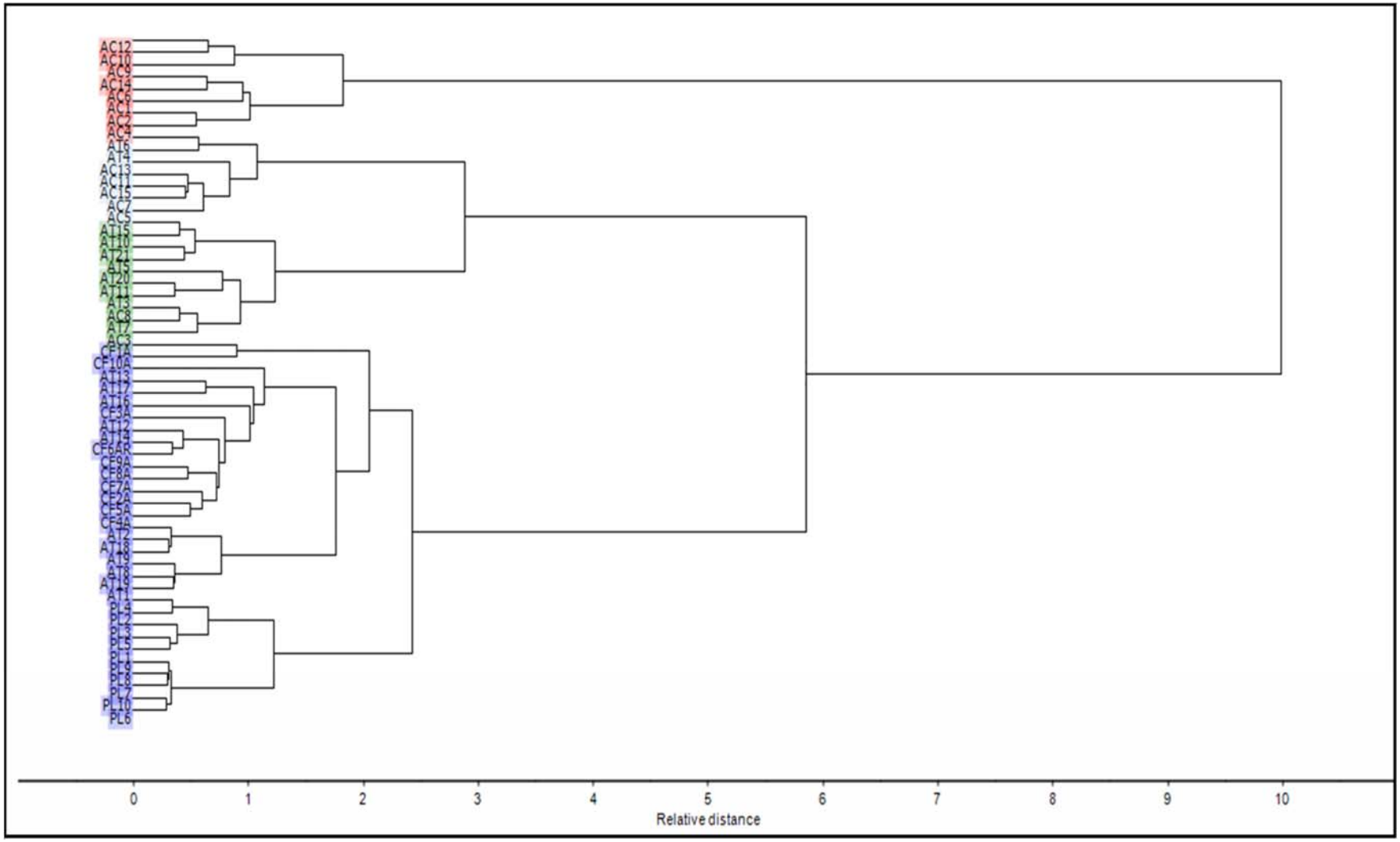


Figure 7.14 Cluster analysis plot for first derivative IR spectra of AMX FM samples, CF samples and PL samples

7.6.3. CONCLUSION FOR AMX STUDY

Various PCA models developed for the three different techniques i.e. NIR, IR and Raman were very successful for assessing the quality of various samples of AMX and could differentiate among different brands of AMX. The PCA model and the HCA dendrogram, for first derivative of NIR spectra proved to be the best as the most clear distinction was possible by PCA as well as for cluster analysis. The Raman and IR-PCA models were found to be somewhat similar in context of the gradient observed in the clusters. The PCA obtained with zero order Raman-PCA was similar to that obtained by SGolay first derivative IR-PCA model. Raman-HCA dendrogram was better than IR-HCA as quite clear classification and correlation could be observed. Both models Raman-PCA and IR-PCA, although, could discriminate the samples but in both models cluster 1A was found to be closer to the CF sample cluster whereas NIR could clearly separate this difference. Although in zero order NIR PCA model cluster 1A was found to be closer to CF clusters 3A and 3C, hence this proved the benefit of using SGolay derivatisation as more clear and well separated clusters were obtained for this study.

7.7. METHOD 7B. STUDY OF THE QUALITY OF AMX-PC BRANDS

7.7.1. MARKETED FORMULATIONS AND LABORATORY SAMPLE PREPARATION

Various film coated tablets containing combination of AMX and PC were bought from local pharmacy stores and were used for analysis. Each tablet consists of 500 mg AMX and 125 mg PC. Total 16 formulations were used for the study of AMX-PC. The various brands undertaken for study are listed in Table 7.13.

Table 7.13. List of the marketed and laboratory formulations of AMX-PC (AMX-PC FM)

Company Name	Quantity of AMX and PC per Formulation (mg)		Sample Code
	AMX	PC	
Megaclav	500	125	AP1
Lab prepared tablet	500	125	*AP2
Amokav	500	125	AP3
Ovimox	500	125	AP4
Omniclav	500	125	AP5
Edmox	500	125	AP6
Clavox	500	125	AP7
MPX-CV	500	125	AP8
AmotidClav	500	125	AP9
Polyclav	500	125	AP10
Moxiforce-CV	500	125	AP11
Moxikind-CV	500	125	AP12
Myclav	500	125	AP13
Augmentin	500	125	AP14
Bactoclav	500	125	AP15
Votclav	500	125	AP16

Underlined samples comprise test set; *Laboratory prepared tablet

Three type of laboratory samples were prepared in the lab (25, 27): (I) Standard tablet of AMX-PC were prepared in laboratory as per the formula mentioned in Table 7.14, (II) Samples with no AMX-PC i.e. Placebo (PL) samples (containing only excipients) as mentioned in Table 7.3 Section 7.7.2. Ten placebo tablet mixtures were prepared in the laboratory and (III) Spurious samples (or counterfeit samples) (CF samples) as per Table 7.15, having varied (low or high) concentration of AMX/PC combined with other drugs such as paracetamol, aspirin, diclofenac, etc. Ten counterfeit tablet mixtures were prepared as mentioned in Table 7.15 in the laboratory.

Table 7.14 Composition of Laboratory prepared AMX-PC tablets (25,27)

Ingredient	AMX-PC Tablet (AMX:PC=4:1)
Amoxicillin trihydrate	573.87 mg
Potassium clavulanate	148.93 mg
Colloidal silica	9 mg
Cross carmellose sodium	40 mg
Magnesium stearate	20 mg
Microcrystalline cellulose	163.2 mg
Cross-povidone	45 mg
Total weight	1000 mg

Table 7.15 Composition of the counterfeit (CF) samples

Ingredients	Amount in mg									
	CF1AP	CF2AP	CF3AP	CF4AP	CF5AP	CF6AP	CF7AP	CF8AP	CF9AP	CF10AP
AMX	50	75	100	150	80	20	70	125	90	100
PC	75	20	50	90	40	80	140	100	30	60
Other additive	100	125	60	75	130	140	70	55	85	90
Cross Carmellose Sodium	25	--	35	--	50	--	60	--	75	--
Sodium Starch Glycollate	--	25	--	--	--	75	--	--	--	--
Magnesium stearate	--	--	--	37.5	--	--	--	--	--	28
Starch	75	0	50	--	25	--	30	--	55	0
Cross Povidone	--	--	--	--	--	--	--	50	--	25
Aspartame	50	--	60	--	75	--	85	--	50	--
Sacharrin	0	75	--	50	--	25	--	35	--	75
Micro crystalline cellulose	0	25	--	37.5	--	50	--	5	--	12
Talc	--	--	25	--	10	--	25	--	--	--
Colloidal silica	5	--	--	--	--	--	--	--	20	--
Mannitol	120	--	120	0	90	--	--	--	--	--
Lactose	--	155	--	60	--	--	20	--	95	--
Dibasic calcium phosphate	--	--	--	--	--	110	--	130	--	110
Total weight	500 mg									

Underlined samples comprise test set

The other additives used were CF1A: Aspirin; CF2A: Paracetamol; CF3A: Diclofenac; CF4A: Atenolol; CF5A: Ramipril; CF6A: Azithromycin; CF7A: Phenylephrine; CF8A: Ranitidine; CF9A: Ibuprofen; CF10A: Cefpodoxime proxetil.

A blinded test set was built up in order to check the model accuracy. The test set comprised of 4 samples viz: AP1, AP4, PL8, CF3AP.

7.7.2. RESULT AND DISCUSSION

7.7.2.1. NIR- chemometric models

7.7.2.1.1. NIR PCA model

Figure 7.15 shows the overlay of NIR spectra of all the AMX-PC samples including FM, CF and PL samples. Figure 7.16 shows the PCA cluster plots for the analysis of AMX-PC samples. The description of PCA clusters has been given in Table 7.16.

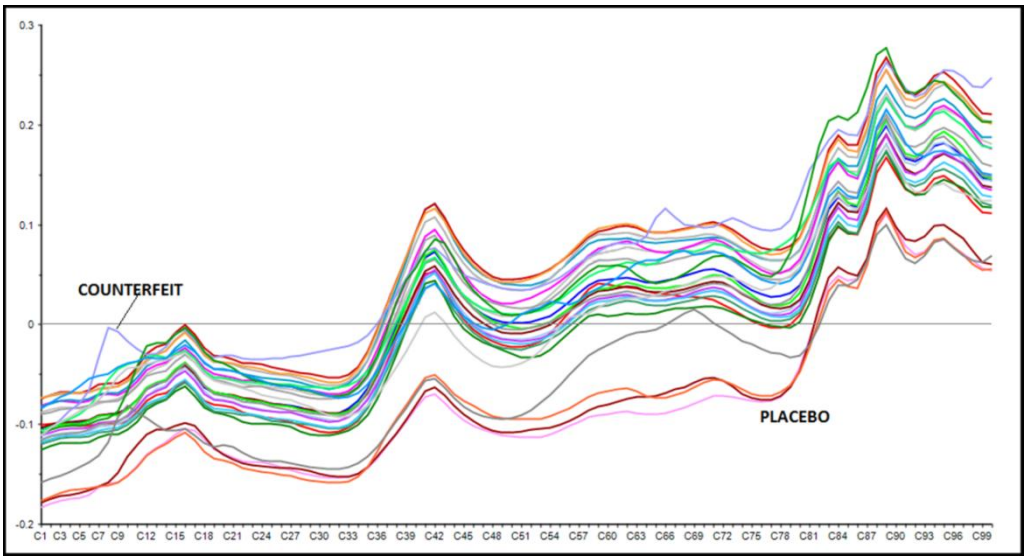


Figure 7.15 Overlay of the zero order NIR spectra of AMX-PC FM samples, CF samples and PL samples

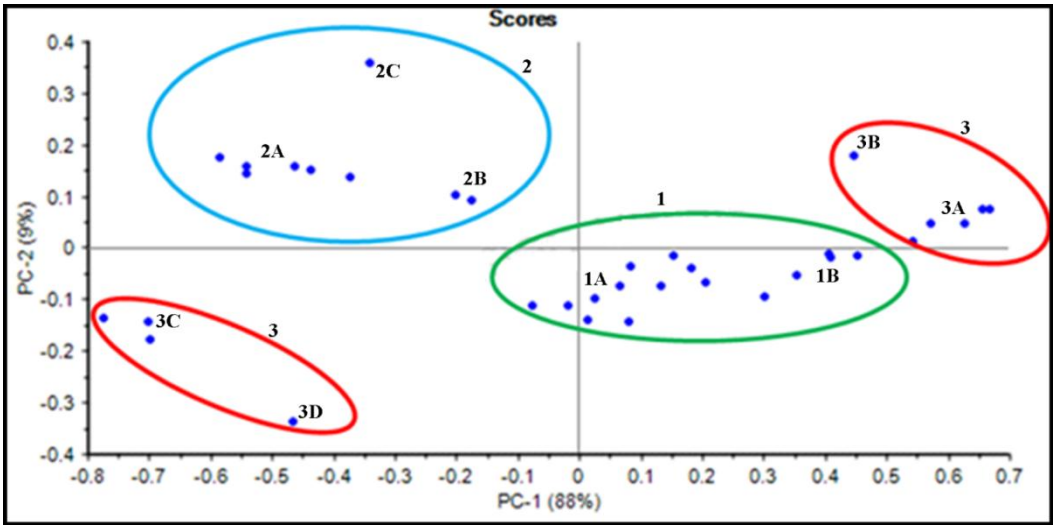


Figure 7.16 PCA plot for the zero order NIR spectra of AMX-PC FM samples, CF samples and PL samples

Table 7.16 Description of clusters formed as per PCA plot in Figure 7.16

Sample	Cluster No.	Samples in the cluster
FORMULATION	1A	AP1, *AP2, AP3, AP4, AP5, AP9-13, AP16
	1B	AP6, AP7, AP8, AP14, AP15
PLACEBO	2A	PL1, PL2, PL3, PL4, PL6, PL8, PL9
	2B	PL5, PL7
	2C	PL10
COUNTERFEIT	3A	CF1AP, CF2AP, CF5AP, CF7AP, CF10AP
	3B	CF4AP
	3C	CF3AP, CF6AP, CF8AP
	3D	CF9AP

The percentage explained variance of the 7 PCs calculated for the model is given in Table 7.17. The PC1 and PC2 showing maximum values were used to construct the PCA plot.

Table 7.17 Explained variance of the PCs

	Percentage Explained variance						
PC	1	2	3	4	5	6	7
CAL	88.02	96.61	98.41	98.87	99.18	99.45	99.65
VAL	87.63	96.41	97.88	98.13	98.25	98.34	98.55

In the PCA plots, four main clusters were found, i.e. one for the AMX-PC FM samples (1 with subclusters 1A and 1B) (green colour), one of PL samples (2) (blue colour), and 4 sub-clusters for CF samples (3A-D) (red colour). The model shows four different clusters and can specifically differentiate the AMX-PC samples from the CF and PL samples. The test set FM samples and the lab prepared tablet sample AP2 were found in cluster 1A which is clearly differtiated from PL and CF sample cluster.

7.7.2.1.2. NIR HCA model

Figure 7.17 shows the hierarchical cluster analysis of all the samples wherein we can find the classification of the different group of samples (FM, CF and PL). In HCA classified one cluster for PL samples, two for CF samples and two for FM samples. The samples in cluster 1A of PCA were found close to that in cluster 3A and 3B and the samples in cluster 1B of PCA were found close to samples of cluster 3C and 3D. The clusters had a very clear cut distinction of the three groups as seen in the HCA dendrogram which showed the appropriateness of the PCA and HCA models for the quality assessment.

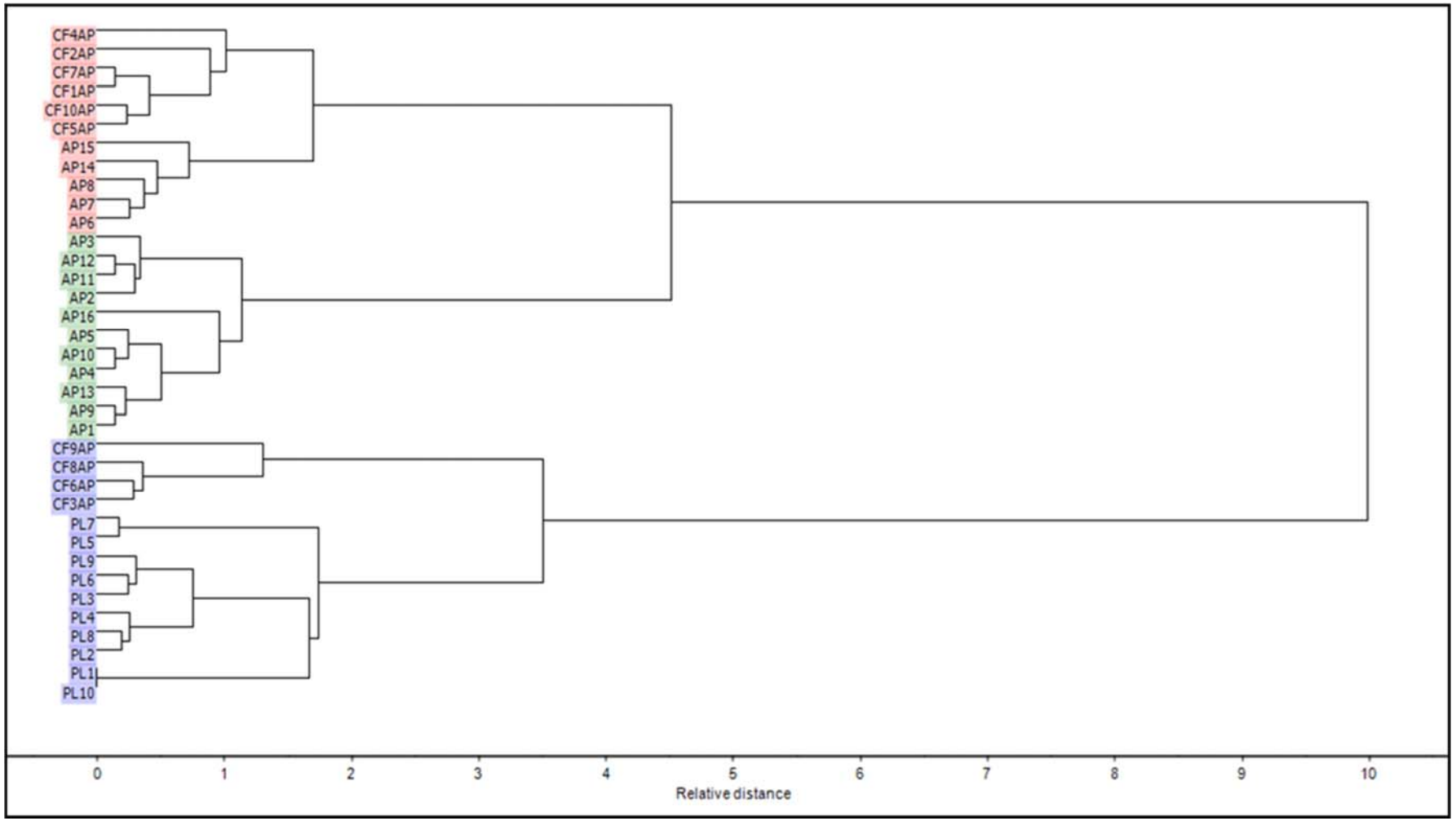


Figure 7.17 Cluster analysis plot for the zero order NIR spectra of AMX-PC FM samples, CF samples and PL samples

7.7.2.2. Raman- chemometric models

7.7.2.2.1. Raman PCA model

Figure 7.18 shows the overlay Raman spectra of all AMX-PC samples including FM, CF and PL samples. The Figure 7.19 shows the PCA cluster plots for the analysis of AMX-PC samples. The description of PCA plot has been given in Table 7.18.

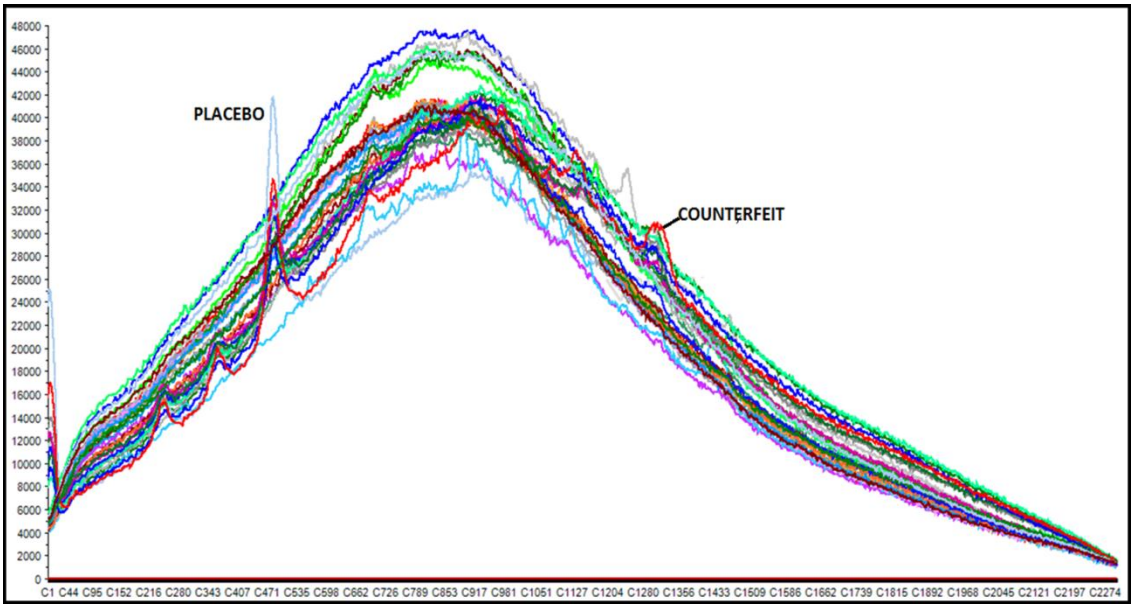


Figure 7.18 Overlay of the zero order Raman spectra of AMX-PC FM samples, CF samples and PL samples

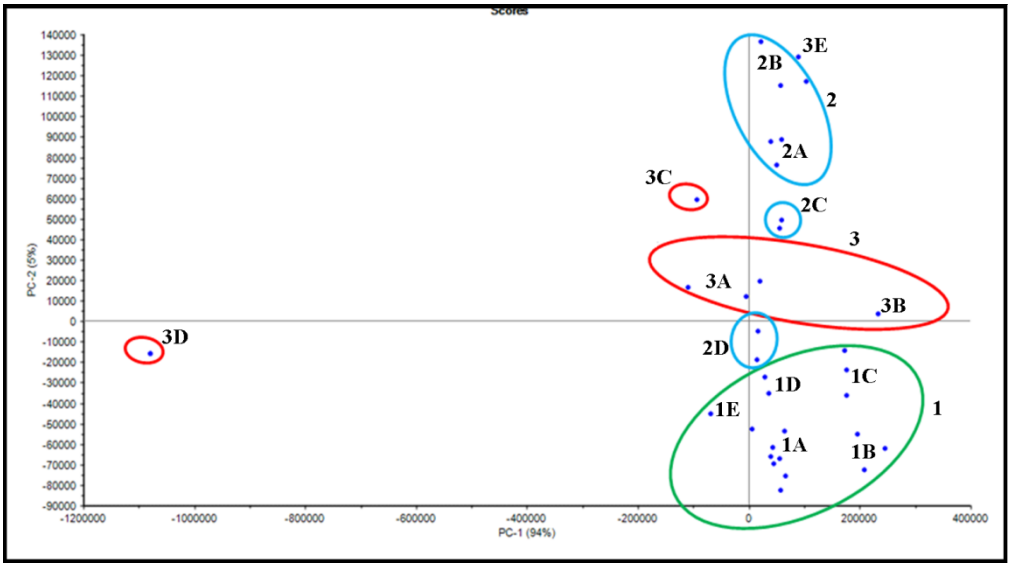


Figure 7.19 PCA plot for the zero order Raman spectra of AMX-PC FM samples, CF samples and PL samples

Table 7.18 Description of clusters formed as per PCA plot in Figure 7.19

Sample	Cluster No.	Samples in the cluster
FORMULATION	1A	AP1, AP9, AP11, AP13, AP14, AP15, AP16
	1B	*AP2, AP4, AP8
	1C	AP3, AP10, AP12
	1D	AP5, AP6
	1E	AP7
PLACEBO	2A	PL1, PL2, PL6
	2B	PL3, PL7, PL8
	2C	PL4, PL5
	2D	PL9, PL10
COUNTERFEIT	3A	CF3AP, CF6AP, CF7AP,
	3B	CF1AP, CF2AP
	3C	CF4AP
	3D	CF9A, CF10AP
	3E	CF5AP

The percentage explained variance of the 7 PCs calculated for the model is given in Table 7.19. The PC1 and PC2 showing maximum values were used to construct the PCA plot.

Table 7.19 Explained variance of the PCs

	Percentage Explained variance						
PCs	1	2	3	4	5	6	7
CAL	93.85	98.98	99.62	99.77	99.83	99.87	99.90
VAL	93.66	98.84	99.44	99.63	99.65	99.68	99.69

The PCA plot showed seven main clusters: (1) one for the AMX-PC FM samples (with five subclusters 1A-E) (green colour), (2) four subclusters of PL samples (2A-D) (blue colour) and (3) five subclusters for CF samples (3A-E) (red colour) as described in Table 7.18. The model could however differentiate the AMX-PC FM samples from the CF and PL samples even though the CF and PL clusters were scattered and the test set samples in the PCA plots were also found to be close to the training set sample clusters. The samples AP4 and AP8 were found to be similar to AP2 (lab prepared).

7.7.2.2.2. Raman HCA model

Figure 7.20 shows the hierarchical cluster analysis of all the samples wherein the classification of the samples into different groups for FM, CF and PL samples was found. Two clusters for FM were found. The CF clusters were found to be scattered as in PCA. One major cluster was found for PL samples except for PL4, PL5 and PL9, PL10 which correlated with the PCA model. The clusters formed in the HCA dendrogram correlate with the PCA clusters.

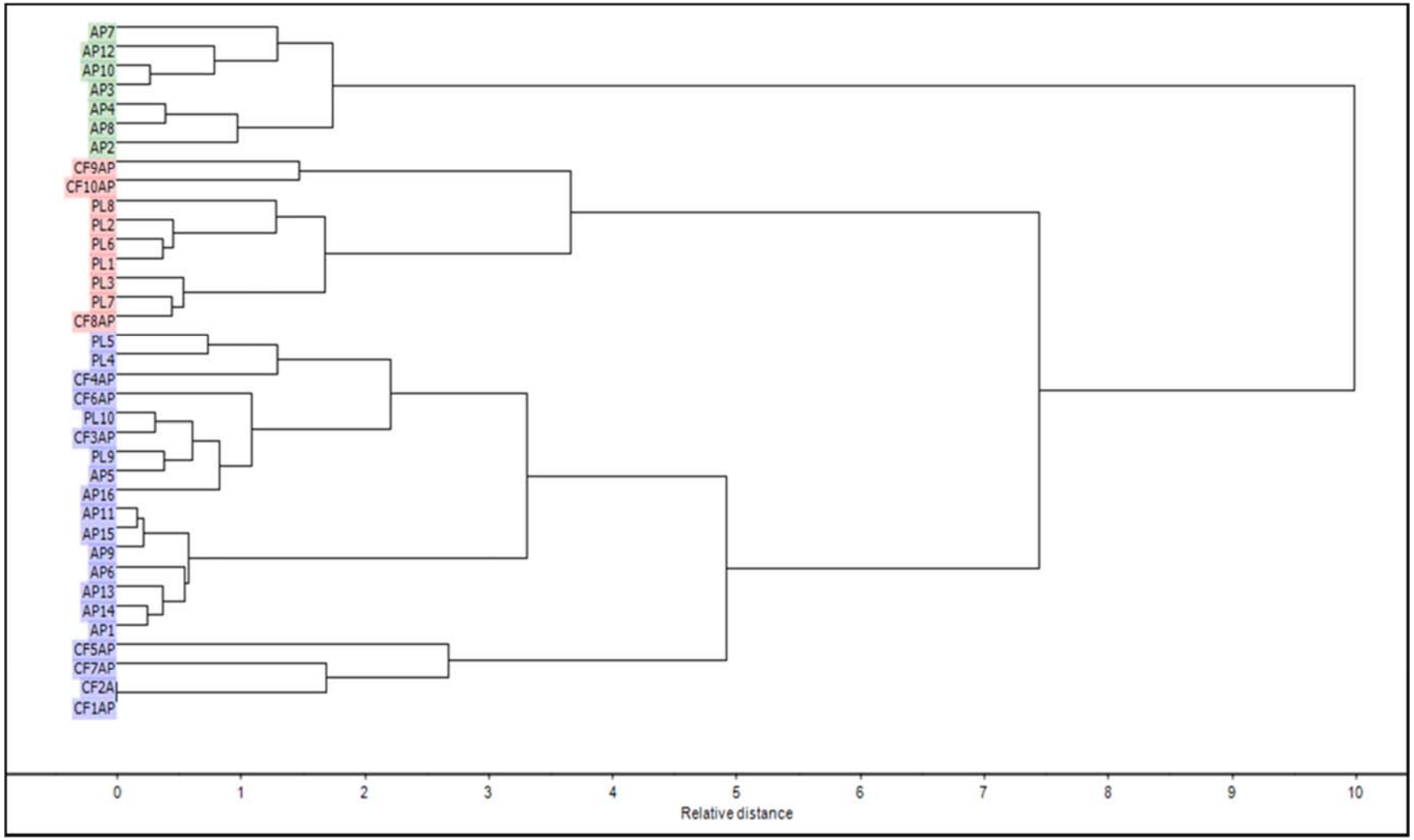


Figure 7.20 Cluster analysis plot for the zero order Raman spectra of AMX-PC FM samples, CF samples and PL samples

7.7.2.3. IR- chemometric models

7.7.2.3.1. IR PCA model

Figure 7.21 shows the overlay IR spectra of all the AMX-PC samples including FM, CF and PL samples. The Figure 7.22 shows the PCA cluster plots for the analysis of AMX-PC samples. The PCA plot clusters have been described in Table 7.20.

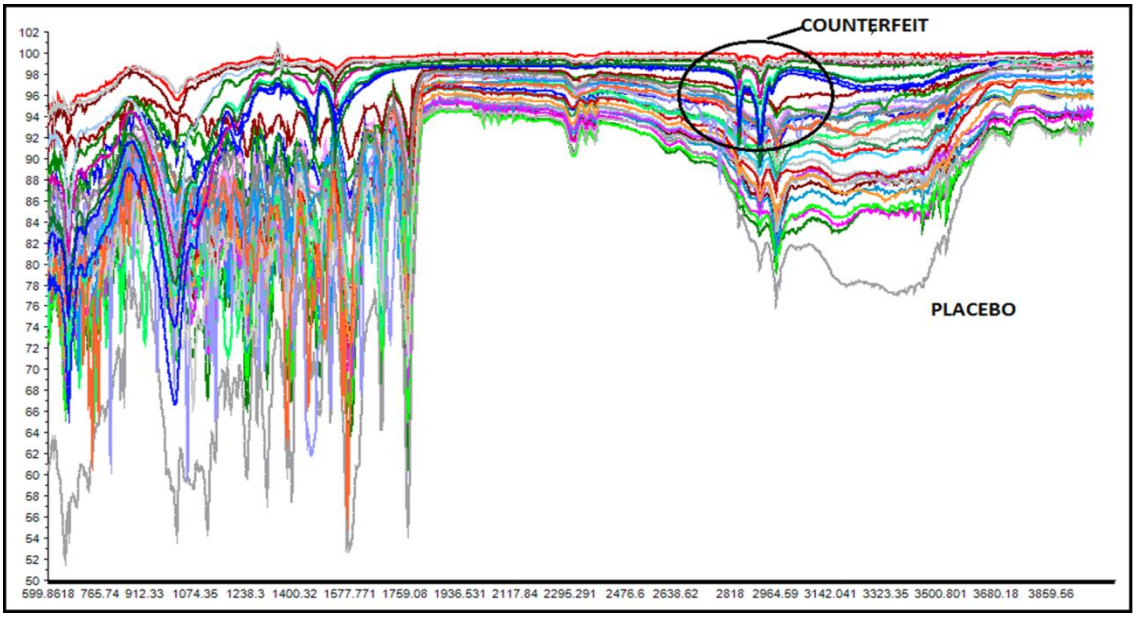


Figure 7.21 Overlay zero order IR spectra of AMX-PC FM samples, CF samples and PL samples

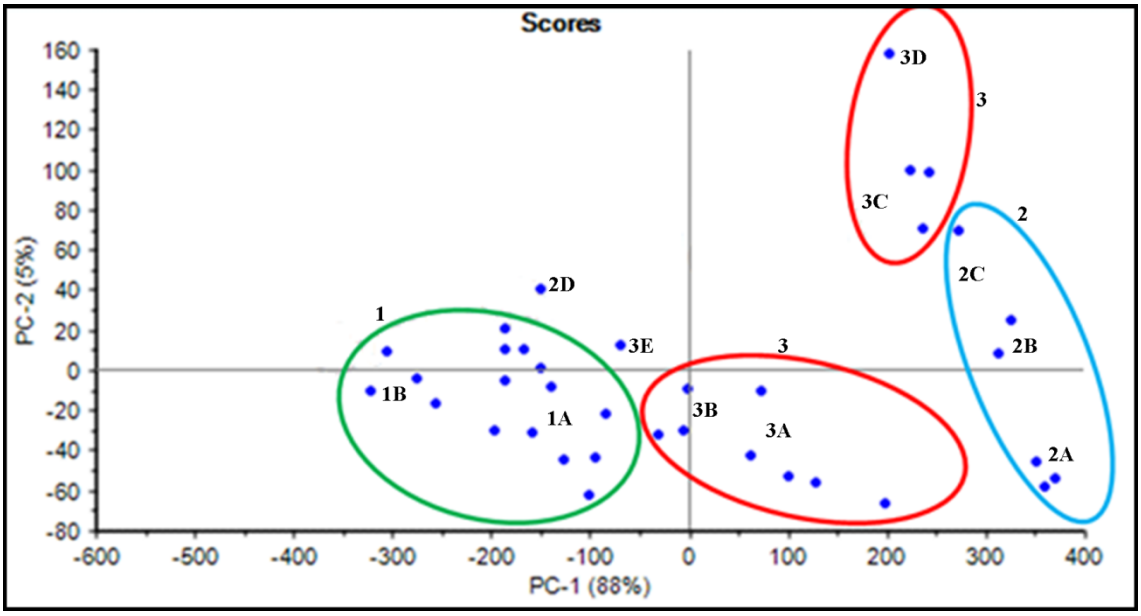


Figure 7.22 PCA plot for the zero order IR spectra of AMX-PC FM samples, CF samples and PL samples

Table 7.20 Description of clusters formed as per PCA plot in Figure 7.22

Sample	Cluster No.	Samples in the cluster
FORMULATION	1A	AP1, *AP2, AP4, AP5, AP6 to AP8, AP10 to AP13, AP16
	1B	AP3, AP9, AP14, AP15
PLACEBO	2A	PL6, PL9, PL10
	2B	PL7, PL8
	2C	PL3
	2D	PL4
COUNTERFEIT	3A	CF3AP to CF5AP, CF7AP, CF10AP,
	3B	PL2, PL5, CF1AP
	3C	PL1, CF2AP, CF9AP
	3D	CF8AP
	3E	CF6AP

The percentage explained variance of the 7 PCs calculated for the model is given in Table 7.21. The PC1 and PC2 showing maximum values were used to construct the PCA plot.

Table 7.21 Explained variance of the PCs

	Percentage Explained variance						
PC	1	2	3	4	5	6	7
CAL	87.94	92.91	95.79	97.27	98.19	98.66	99.00
VAL	86.88	91.86	92.44	92.84	93.38	93.95	94.15

The PCA plots showed four main clusters i.e. (1) one for the AMX-PC FM samples (green colour), (2) four subclusters of PL samples (2A-D) (blue colour), five subclusters for CF samples (3A-E) (red colour). The cluster of IR-PCA model was found to be less scattered than Raman-PCA model. 1A was the major FM cluster which included the lab sample AP2 and two test FM samples. The clusters 3B and 3C were the mixture of PL and CF samples. However the model could specifically differentiate the AMX-PC FM samples from the CF and PL samples. The test set samples in the PCA plots were found to be close to the training set sample clusters and present in the major cluster 1A.

7.7.2.3.2. IR HCA model

The Figure 7.23 shows the cluster analysis of all the samples wherein a clear classification of all the three sample groups was found which correlated with the PCA plot. Two significant clusters were formed, i.e. one cluster for all the FM samples including three PL samples (PL2, PL4, PL5) and another comprising a mixture of CF and PL samples. However the FM sample cluster was quite clear and distinctly observed.

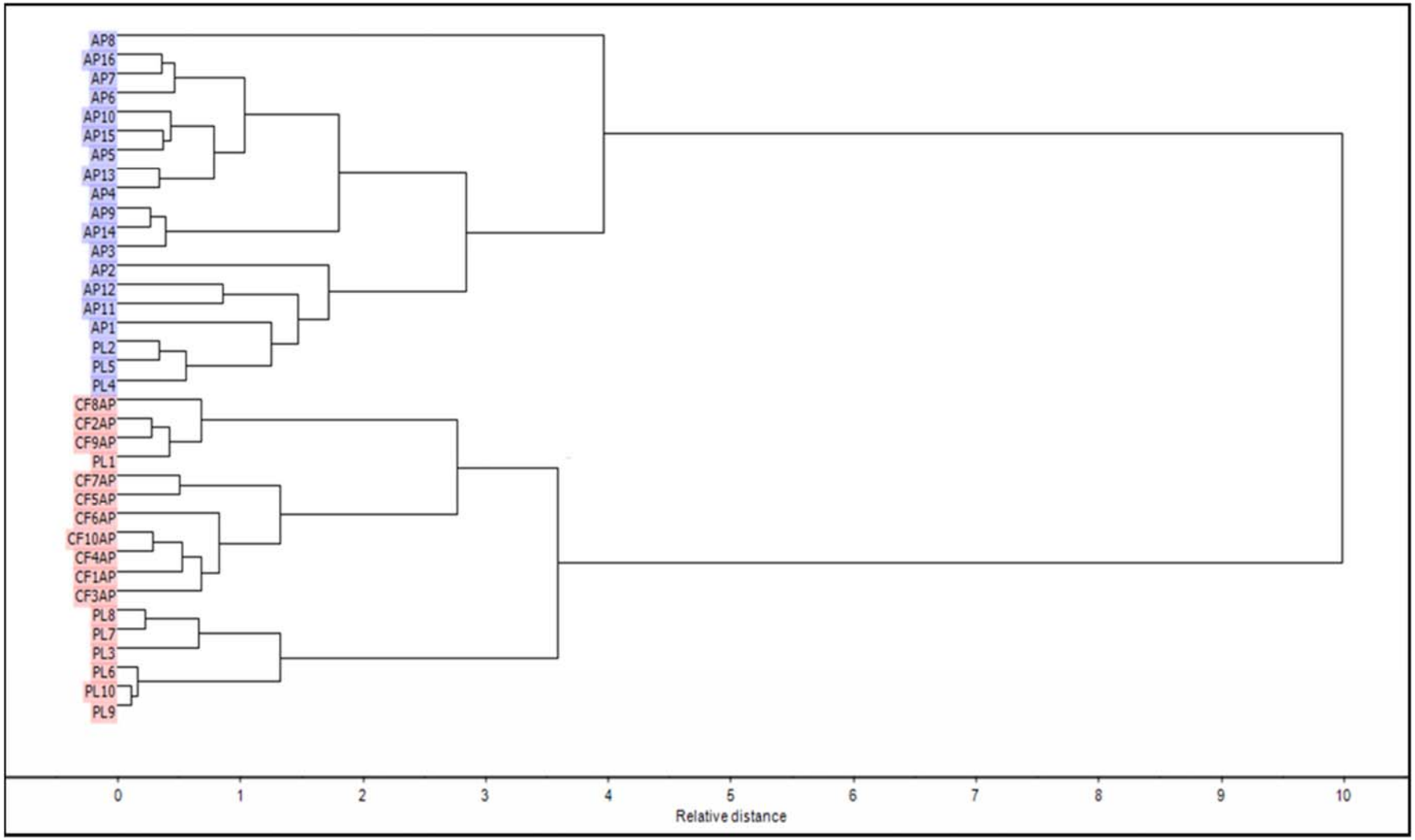


Figure 7.23 Cluster analysis plot for the IR spectra of AMX-PC FM samples, CF samples and PL samples

7.7.3. CONCLUSION FOR AMX-PC STUDY

The various PCA models developed for the three different techniques i.e. NIR, IR and Raman were successful for assessing the quality of various samples of AMX. The PCA model for zero order NIR spectra and zero order IR spectra proved to be the best as the most clear distinction was possible by PCA as well as for cluster analysis. The Raman PCA plot could not specifically differentiate the FM samples from the CF and PL ones. The HCA classification was quite clear and distinct in all the three models but the best results were obtained for NIR and IR model. The PCA models developed from SGolay derivatisation caused the merging of FM samples with the CF samples hence could not be beneficial in this case.

7.8. CONCLUSION

The qualitative assessment of different brands of AMX was carried out with three analytical techniques i.e. IR, NIR and Raman spectroscopy. The samples were analysed and statistical chemometric models i.e. PCA and HCA were built up alongwith the laboratory prepared counterfeit and placebo samples. The models thus developed could differentiate the formulation samples from the placebo and counterfeit samples and thus can be applied for quality assessment of SFFC drugs in future.

In case of AMX study, the NIR and Raman models were comparatively better and showed better differentiation among various samples than the IR model. In case of AMX-PC study, the IR and NIR methods were found to give good results as compared to the Raman method. From the models, mislabelling of brands can be detected as a wide variety of counterfeits were under taken for the study. All the models showed fakes as outliers. The models developed were exclusively qualitative and further studies in developing more advanced chemometric models are needed. However absence of AMX could be easily detected by any of these models.

Physical examination is the preliminary step for detection of visual inspection of SFFC. The detection of counterfeiting on quantitative basis is done by HPLC. But if some other drug is present, it may or may not be detected, so HPLC may not be feasible in that case. Hence the models developed are purely qualitative. Generally

only NIR and Raman are well-known techniques for assessment of the quality of samples. NIR is usually preferable over Raman data and gives robust models. The study also showed that the simple IR technique can also be fruitful for qualitative assessment and thus the analysis can be worked out even without the sophisticated techniques like NIR and Raman. However NIR and Raman have their own advantages of being more specific. Especially, for this method the NIR technique was found to be the best for both the studies. Sometimes library of the spectra needs to be created for the success of this model which forms the spectral signature of the compound which is specific for that particular compound. Hence it is suggested for future studies, that, more supervised models can be developed as the adulteration of AMX would be difficult to detect by a simple PCA technique.

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