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MEDICAL PHYSICS

ON SIMILARITIES IN INFRARED SPECTRA OF COMPLEX DRUGS

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Abstract. A chromatographic separation of active ingredients of Combivir, Epivir, Kaletra, Norvir, Prezista, Retrovir, Trivizir, Valcyte, and Viramune is performed on thin layer chromatography. The spectra of these nine drugs were recorded using the Fourier transform infrared spectroscopy. This information is then analyzed by means of the cosine correlation. The comparison of the infrared spectra in the perspective of the adopted similarity measure is possible to visualize with present day computer tools, and the emerging clusters provide additional information about the similarities of the investigated set of complex drugs.

Key words: Chromatographic separation, Combivir, Epivir, Kaletra, Norvir, Prezista, Retrovir, Trivizir, Valcyte, Viramune, visualization.

1. INTRODUCTION

Mathematical modelling of complex processes is a major challenge for contemporary scientists. One of major role in this picture is playing by the fractional calculus and its applications [1–9]. The signal analysis of complex systems by using new methods and techniques started to reveal hidden aspects of them [10–13]. A still open problem is to elaborate new fractional dynamics models for complex systems regarding the high effective therapy for acquired immunodeficiency syndrome. During the last decades, high effective therapy for acquired immunodeficiency syndrome (AIDS) was reported by using commercial complex mixtures comprising different combinations of several anti-HIV drugs including nucleoside reverse transcriptase

inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) or protease inhibitors (PIs).

Norvir and Kaletra contain Lopinavir, which is developed from Ritonavir. They belong to a class of drugs called PIs, suppress plasma viral load and enhance immunological status in therapy naive and experienced patients with HIV-1 infection [14]. Activity was assessed in vitro in acutely infected lymphoblastoid cell lines and in peripheral blood lymphocytes [15]. Combination of Lopinavir with Ritonavir reduces the HIV RNA Level in Cerebrospinal Fluid [16].

Combivir [17] and Kaletra [18] are an HIV medication, called NRTIs and PIs, respectively. They prevent HIV by increasing the number of CD4 cells, which prevent the cells from producing new virus and decrease the amount of virus in the body.

Valganciclovir is used for the prevention of cytomegalovirus (CMV) disease and treating other opportunistic infections of HIV infection. To our knowledge Valganciclovir is the first antiviral agent shown to reduce HHV-8 replication in vivo [19]. Valganciclovir also has known antiviral activity against HHV-6 [20].

Valcyte [21–23] and Nevirapine (NVP) [24, 25], are non-nucleoside reverse transcriptase inhibitor (NNRTI) and have synergistic effect on inhibition of the human immunodeficiency virus (HIV-1). They decrease MTCT because their lipophilic and easily absorbed [25]. Valcyte has been used for the treatment of CMV retinitis in patients with weakened immune systems, it has been used in various prophylaxis protocols to reduce risk of perinatal HIV transmission [26] and given to breast feeding infants of HIV-infected mothers to prevent HIV transmission via breast milk [27], but Nevirapine used in the highly active antiretroviral treatment (HAART) which is considered as one of the most significant advances in the field of HIV therapy [28].

Having in mind the importance of these nine drugs for the medical purposes, we compare the spectra of the drugs by means of the cosine correlation. This measure establishes a 9×9 matrix of comparison of all pairs of drugs, providing information that can be visualized by means of modern computer tools. In our case we shall adopt both 2-dimensional trees and 3-dimensional Multidimensional Scaling (MDS) methods.

In this line of thought the manuscript is organized as follows. Section 2 describes in details the experimental part. In Section 3 we perform the comparisons of the spectra (transmitance versus wavenumber and we find the emerging clusters of the analyzed drugs. Finally, Section 4 presents the main conclusions of this paper.

2. EXPERIMENTAL STUDIES

2.1. MATERIALS

Norvir and Kaletra were kindly provided by Abbott; Viramune by Boehringer Ingelheim and Valcyte by Roche. Epivir, Retrovir, Combivir and Trizivir were kindly provided by GlaxoSmithKline, Prezista was kindly provided by Janssen-Cilag. Analytical grade methanol (BDH, England), analytical grade chloroform (Sigma-Aldrich, Germany), analytical grade n-hexane (BDH, England) were also used during the method development.

2.2. SAMPLE SOLUTION PREPARATION

Twenty tablets of Combivir: (containing 150 mg of Lamivudine and 300 mg of Zidovudine) or Kaletra: (containing 200 mg of Lopinavir and 50 mg of Ritonavir) or Trizivir: (containing 150 mg of Lamivudine, 300 mg of Abacavir and 300 mg of Zidovudine) were accurately weighed and powdered. A portion of the powder equivalent to 75 mg of Lamivudine and 150 mg of Zidovudine (Combivir) or 100 mg of Lopinavir and 25 mg of Ritonavir (Kaletra) or 75 mg of Lamivudine, 150 mg of Abacavir and 150 mg of Zidovudine (Trizivir) was weighed and quantitatively transferred in to a 100 ml volumetric flask. The powder was dissolved using Ethanol with the aid of mechanical shaking for 15 min and sonication for 30 min for separating out the insoluble materials and binding agents. A sample solution of 25 ml of the stock solution to a 100 ml volumetric flask and diluted with Ethanol of the stock solution to a 100 ml volumetric flask and diluted with Ethanol.

2.3. CHROMATOGRAPHIC SEPARATION

The chromatographic separation was performed on thin layer chromatography (TLC) 10 cm $\times$ 20 cm aluminum plates coated with 0.2 mm layers of silica gel 60F_{254} (E. Merck, Darmstadt, Germany) with fluorescent indicator. Samples were applied to the plates. The origin and mobile phase line are marked using a soft pencil on silica gel 60 F_{254} layer (called a "chromatoplate"), and disposable 2 μL glass micropipet are used to spot the standards and sample 1.5 cm up from the bottom edge. The bands were dried, then placed in a glass Jar and is slowly solvated by a "mobile phase". The solvent used as mobile phase in the TLC was n-hexane: chloroform: methanol (1:7:2,v/v/v) for Combivir, n-hexane: chloroform: methanol(1:6:3, v/v/v) for Kaletra and n-hexane: chloroform: methanol (1:5:4,v/v/v) for Trizivir after that linear ascending was development for 25 min at room temperature (25 °C).

The chromatoplate is removed, and the mobile phase is evaporated and were visualized under 254 nm ultraviolet (UV) light.

FTIR spectra of nine drugs, namely Viramune, Valcyte, Norvir, Kaletra, Epivir, Retrovir, Combivir, Trivizir and Prezista were analyzed by using Shimadzu SSU-8000 Fourier Transform Spectrophotometer by KBr disc method. The procedure consisted of dispersing a sample (drug alone) in KBr and compressing into disc by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained by scanning at between 400 and 4,000 cm^{-1}.

2.3.1. FTIR Data for Norvir (Ritonavir)

The FTIR spectrum of Norvir showed sharp characteristics bands at υ=3484.82 cm^{-1} for N-H stretching amide group, a band peak at 1723.18 cm^{-1} owing to the carbonyl group of ester linkage, the carbonyl group (C=O stretching) present in the amide group at 1686.73 cm^{-1}. One band in 2964.35 cm^{-1} is assigned to hydrogen-bonded acid within the molecule. Peaks at 1645.87, 1621.57, and 1530.23 cm^{-1} owing to (–CC– stretching aromatic carbons) present in the benzene ring.

2.3.2. FTIR Data for Viramune (Nevirapine anhydrate)

The FTIR spectrum of Nevirapine showed sharp characteristics peaks at N-H Stretching and C=O at 3284.34 and 1654.58 cm^{-1} of cyclic amide of 7-membered ring in Nevirapine, respectively. Also CH stretch peak at 2950.93 cm^{-1} and a band peak at 1450.49 cm^{-1} owing to C=C stretching (pyridine ring).

2.3.3. FTIR Data for Valcyte (Valganciclovir hydrochloride)

The characteristics peak of the hydroxyl group (OH stretching) at 3443.27 cm^{-1}, a band peak at 3395.30 cm^{-1} owing to amino group (NH_2 stretching), the characteristics peak of the NH stretching imino group at 3480.28 cm^{-1}, a band peak at 1730.58 cm^{-1} owing to carbonyl group of ester linkage but the characteristics peak of the carbonyl group (C=O stretching) appear at 1663.64 cm^{-1}. Peaks at 1294.72 and 1152.89 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system.

2.3.4. FTIR Data for Kaletra (Lopinavir-Ritonavir)

The characteristics peak of the hydroxyl group (OH stretching) at 3436.22 cm^{-1}, a band peak at 3399.33 cm^{-1} owing to imino group (N-H stretching), the characteristics peak of the carbonyl group (C=O stretching) present in the amide group at 1653.35 cm^{-1}, a band peak at 1450.49 cm^{-1} owing to C=C stretching (aromatic) confirm the presence of Lopinavir. Peaks at 1290.42 and 1150.82 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system.

For Ritonavir the characteristics peak of the N-H stretching amide group at 3484.82 cm^{-1}, a band peak at 1723.18 cm^{-1} owing to the carbonyl group of ester

linkage, the carbonyl group (C=O stretching) present in the amide group at 1686.73 cm^{-1}. One band in 2964.35 cm^{-1} is assigned to hydrogen-bonded acid within the molecule. Peaks at 1645.87, 1621.57, and 1530.23 cm^{-1} owing to (–C=C– stretching aromatic carbons) present in the benzene ring.

2.3.5. FTIR Data for Epivir (Lamivudine)

The characteristics peak of the hydroxyl group (OH stretching) at 3440.24 cm^{-1}, a band peak at 3392.38 cm^{-1} owing amino group (NH_2 stretching), the characteristics peak of the carbonyl group (C=O stretching) present in the cystedine nucleus at 1652.15 cm^{-1}, a band peak at 1454.66 cm^{-1} owing C=C stretching (aromatic) confirm the presence of Lamivudine. Peaks at 1278.23 and 1155.47 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system present in the Oxathiolane ring.

2.3.6. FTIR Data for Retrovir (Zidovudine)

For Zidovudine the characteristics peak of the hydroxyl group (OH stretching)at 3466.85 cm^{-1}, a band peak at 2130.16 cm^{-1} owing azide group (-N_3 stretching), the carbonyl group (C=O stretching) present in the amide group at 1681.55 cm^{-1}, a band peak at 1680.16 cm^{-1} owing imino group (N-H stretching), a band peak at 1465.16 cm^{-1} owing C=C stretching (aromatic). One band in 1374 cm^{-1} is assigned to CH_2. Peaks at 1279.51 and 1170.55 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system present in the oxalane ring.

2.3.7. FTIR Data for Combivir (Lamivudine- Zidovudine)

The characteristics peak of the hydroxyl group (OH stretching) at 3440.24 cm^{-1}, a band peak at 3392.38 cm-1 owing amino group (NH_2 stretching), the characteristics peak of the carbonyl group (C=O stretching) present in the cystedine nucleus at 1652.15 cm^{-1}, a band peak at 1454.66 cm^{-1} owing C=C stretching (aromatic) confirm the presence of Lamivudine. Peaks at 1278.23 and 1155.47 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system present in the oxathiolane ring. For Zidovudine the characteristics peak of the hydroxyl group (OH stretching)at 3466.85 cm^{-1}, a band peak at 2130.16 cm^{-1} owing azide group (-N_3 stretching), the carbonyl group (C=O stretching) present in the amide group at 1681.55 cm^{-1}, a band peak at 1680.16 cm^{-1} owing imino group (N-H stretching), a band peak at 1465.16 cm owing C=C stretching (aromatic). One band in 1374 cm^{-1} is assigned to CH_2. Peaks at 1279.51and 1170.55 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system present in the oxalane ring.

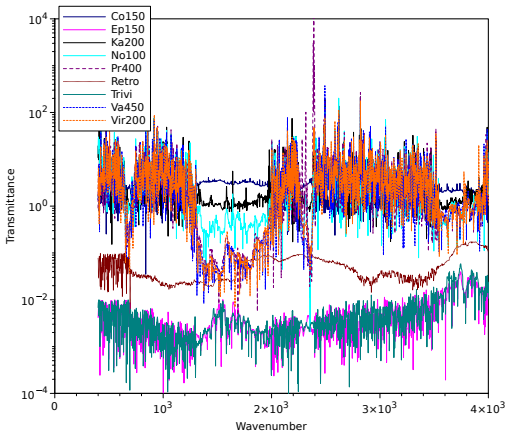

Fig. 1 – Transmittance versus Wavenumber (in cm^{-1}) for the {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200.}

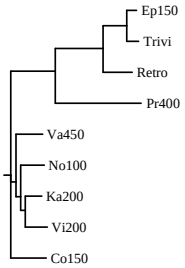

Fig. 2 – Drawgram using squared cosine correlation r^2_{cc} for the {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200.}

2.3.8. FTIR Data for Trizivir (Lamivudine- Zidovudine- Abacavir)

The procured sample of Trizivir was tested for its identification by the FT-IR spectra of the physical mixture which exhibited absorption peaks similar to those of the pure drug sample. The characteristics peak of the hydroxyl group (OH stretching) at 3440.24 cm^{-1}, a band peak at 3392.38 cm^{-1} owing amino group (NH_2 stretching), the characteristics peak of the carbonyl group (C=O stretching) present in the cystedine nucleus at 1652.15 cm^{-1}, a band peak at 1454.66 cm^{-1} owing C=C stretching (aromatic) confirm the presence of Lamivudine. Peaks at 1278.23 and 1155.47 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system present in the oxathiolane ring. For Zidovudine the characteristics peak of the hydroxyl group (OH stretching)at 3466.85 cm^{-1}, a band peak at 2130.16 cm^{-1} owing azide group (-N_3 stretching), the carbonyl group (C=O stretching) present in the amide group at 1681.55 cm^{-1}, a band peak at 1680.16 cm^{-1} owing imino group (N-H stretching),

band peak at 1465.16 cm^{-1} owing C=C stretching (aromatic). One band in 1374 cm^{-1} is assigned to CH_2. Peaks at 1279.51and 1170.55 cm^{-1} owing to asymmetrical and symmetrical stretching of C-O-C system present in the oxalane ring. The following FTIR characteristic peaks at 3232.7 cm^{-1}, 3129.8 cm^{-1} for N-H and O-H stretch while C-H stretch is observed at 2877.8 cm^{-1}, peak for C=C stretch of cyclopentene ring is observed at 1620.0 cm^{-1} and at 1458.7 cm^{-1} for C-H bending (CH_2 scissoring) vibration of C-H bond of monosubstituted cyclopropyl ring, respectively, confirm the presence of Abacavir.

2.3.9. FTIR Data for Prezista (Darunavir)

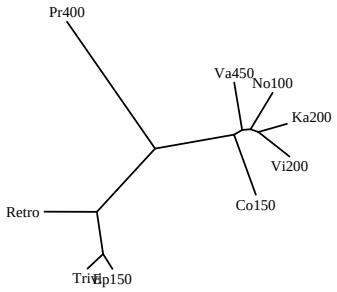

Fig. 3 – Drawtree using squared cosine correlation r_{cc}^2 for the {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200.}

For Prezista the characteristics peak of the hydroxyl group (OH stretching) at 3456.35 cm^{-1}, a band peak at 3382.58 cm^{-1} owing to amino group (NH_2 stretching), another band peak at 3376.93 cm^{-1} owing to imino group (N-H stretching), the characteristics peak of the carbonyl group (C=O stretching) present in the amide group at 1668.15 cm^{-1}, a band peak at 1473.60 cm^{-1} owing C=C stretching (aromatic). Peaks at 1263.41 and 1168.34 cm^{-1}owing to the stretching of C-O-C system.

3. RESULTS AND DISCUSSION

Let us denote by $x(\upsilon)$ the signal representing the transmittance, where υ denotes the wavenumber. In the following charts is adopted the labelling {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200} for {Combivir, Epivir, Kaletra, Norvir, Prezista, Retrovir, Trivizir, Valcyte, Viramune}.

Figure 1 shows transmittance versus wavenumber for the 9 drugs.

For comparing the j-th and k-th signals are adopted the squared cosine correlation r_{cc}^2 and the Jensen-Shannon distance d_{JS} [29, 30] given by:

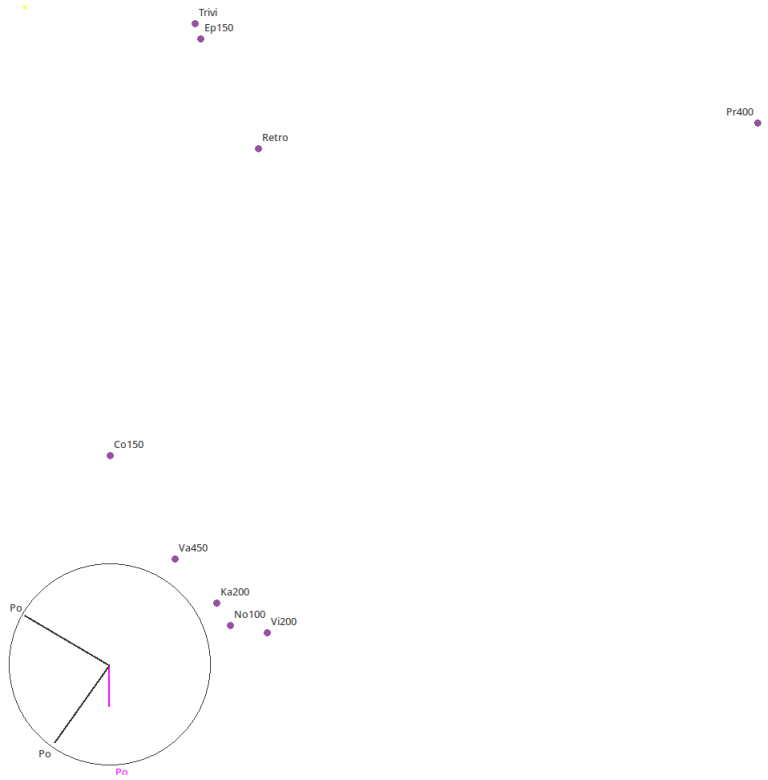

Fig. 4 – MDS using squared cosine correlation r_{cc}^2 for the {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200.}

$$r_{cc}^2(j,k) = \frac{\left[\sum_{i=1}^{n} x_j(\upsilon_i)\, x_k(\upsilon_i)\right]^2}{\sum_{i=1}^{n} x_j^2(\upsilon_i) \sum_{i=1}^{n} x_k^2(\upsilon_i)} \tag{1}$$

$$d_{JS}(j,k) = \frac{1}{2}\sum_{i=1}^{n}\left\{ x_j(\upsilon_i) \ln\left[\frac{2x_j(\upsilon_i)}{x_j(\upsilon_i)+x_k(\upsilon_i)}\right] + x_k(\upsilon_i)\ln\left[\frac{2x_k(\upsilon_i)}{x_j(\upsilon_i)+x_k(\upsilon_i)}\right]\right\} \tag{2}$$

where υ_i denotes the i-th wavenumber and n represents the total number of sampling points.

For visualization were adopted the packages PHYLIP [31] and GGobi [32]. PHYLIP constructs several types of 2-dimensional trees [31]. In the sequel is considered the algorithm “Neighbor”, that constructs a tree by successive clustering of lineages, setting branch lengths as the lineages join, with the visualization options “Drawgram” and “Drawtree”. On the other hand, GGobi with tool “ggvis” constructs

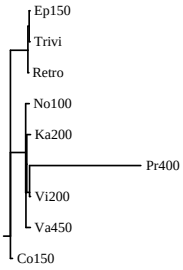

Fig. 5 – Drawgram using Jensen-Shannon distance d_{JS} for the {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200.}

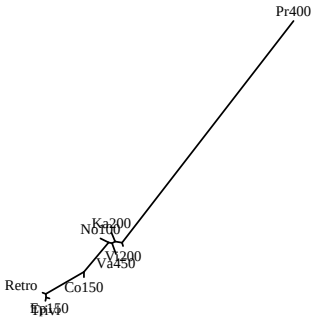

Fig. 6 – Drawtree using Jensen-Shannon distance d_{JS} for the {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200.}

3-dimensional Multidimensional Scaling (MDS) plots [33–42].

Figures 2 and 3 depict the 2-dimensional trees and Figures 4 represents a screen shot of the 3-dimensional MDS plot generated using the index r_{cc}^2 formulated in (1). We observe that the conclusions are similar and that the MDS plot is slightly superior in visualizing the different clusters. Figures 5, 6 and 7 show, correspondingly, the same type of charts when adopting the index d_{JS} defined in (2). We observe results that lead to conclusions close to those obtained previously, confirming the rightness of the measuring indices and the efficiency of the visualization tools.

In all charts it is clear the emergence of several clusters. Nevertheless, there is a slight difference between the charts resulting from the two indices. For the squared cosine correlation (1) we observe three main clusters {Co150, Ka200, No100, Va450, Vi200} (with Co150 slightly apart), {Ep150, Retro, Trivi} (with Retro slightly apart) and {Pr400}.

For the Jensen-Shannon distance (2) we observe four main clusters {Ka200, No100, Va450, Vi200}, {Retro, Trivi}, {Co150, Ep150} and {Pr400}. The slight differences results from the distinct "perspectives" defined by each characterizing index. The adoption of one index over the other depends on the user to decide based his

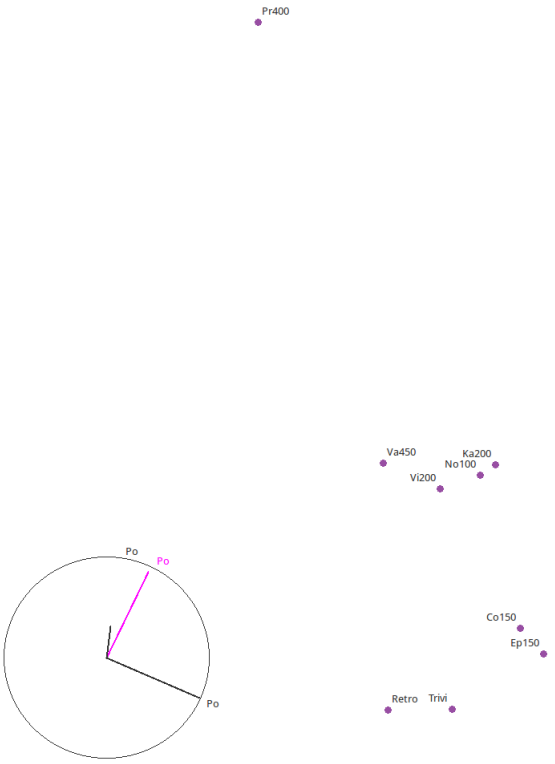

Fig. 7 – MDS using Jensen-Shannon distance d_{JS} for the {Co150, Ep150, Ka200, No100, Pr400, Retro, Trivi, Va450, Vi200.}

own experience. On the other hand, the general conclusions are similar and demonstrate that we can embed measuring indices into computer visualization tools to have a superior quantitative observation of the properties of the objects under study.

4. CONCLUSIONS

FTIR spectra of nine drugs were analyzed with the help of Shimadzu SSU-8000 Fourier Transform Spectrophotometer by KBr disc method. The signal of the spectra reveals the existence of clusters. Our work provides detailed analysis of four important HIV drugs families like (a)- Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs) Anti-Virals, i.e. Trizivir: Abacavir/Lamivudine/Zidovudine, Combivir: Lamivudine/Zidovudine, Epivir: Lamivudine and Retrovir: Zidovudine. (b)- Protease Inhibitors (PIs) Anti-Virals: Kaletra: Lopinavir/Ritonavir, Norvir: Ritonavir, Prezista: Darunavir. (c)- Non-Nucleoside Reverse Transcriptase Inhibitors

(NNRTIs) Anti-Virals, i.e. Viramune Nevirapine. (d)- Herpes Treatments/CMV Disease Anti-Virals: Valcyte: Valganciclovir. The structure-function relationship of each drug is compatible with our results as seen in all Figures, due to the common active ingredients between them or their mechanism of action.

The methods presented in this manuscript to identify the clusters in infrared spectra of nine investigated drugs are rapid, reliable and easy to apply.

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