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Formation of Hydrochlorothiazide – Para-aminobenzoic Acid Cocrystals by Solvent Evaporation Method

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ABSTRACT

Hydrochlorothiazide is a diuretic drug used for mild to moderate hypertension were classified in Class II BSC. The purpose of this study was to explore the formation of hydrochlorothiazide-para-aminobenzoic acid cocrystal by solvent evaporation method. Cocrystals are prepared with a molar ratio of 1:0; 1:1; 1:2; and 2:1 between hydrochlorothiazide and para-aminobenzoic acid. The cocrystals were characterized by Scanning Electron Microscopy (SEM), X-ray Diffractometry (XRD), Differential Scanning Calorimetry (DSC), and Fourier Transform Infrared (FT-IR) spectrophotometer. The hydrochlorothiazide-para-aminobenzoic acid cocrystal has new crystalline peaks at 2θ of 14.904°; 15.41°; 25.553°; 26.5°; 29.844°; 31.083° indicating the formation of a new crystalline phase. The cocrystal showed the melting point at 188.57°C which is different from the initial components. The FTIR spectra of cocrystal showed the shifting of absorption peaks of groups of initial components indicating of formation of hydrochlorothiazide-para-aminobenzoic acid cocrystal through intermolecular hydrogen bond interactions between amine/sulfonamide group and carboxyl group.

KEYWORDS : hydrogen bond, diuretic, BSC, para-aminobenzoic acid

INTRODUCTION

Hydrochlorothiazide is a diuretic drug used for mild to moderate hypertension. According to BCS (Biopharmaceutical Classification System), hydrochlorothiazide is the II class of drugs, namely drugs with low solubility and high permeability (Sanphui, et al., 2015);, causes poor oral absorption. Because it is poorly soluble, hydrochlorothiazide can cause problem of limited dissolution and absorption rates and therefore, improvement of solubility is needed to increase absorption and bioavailability. Hydrochlorothiazide (6-chloro-1,1-dioxo-3,4-dihydro-2H-1λ⁶,2,4-benzothiadiazine-7-sulfonamide) is a weak basic compound (pKa =7.9) (PubChem, 2021). This is consistent with the FDA's statement that another advantage of co-crystals is that they provide a variety of solid-state forms for APIs that lack ionizable functional groups, which are prerequisites for salt formation (FDA, 2018).

Increasing the solubility of an active pharmaceutical ingredient can be done through modification of the crystal structure, such as cocrystal formation techniques with suitable co-formers (Karimi-Jafari, Padrela, Walker, & Croker, 2018). Cocrystals are a class of multicomponent solids consisting two or more different molecular components (Gadade & Pekamwar, 2016) in a single homogenous crystalline phase with well-

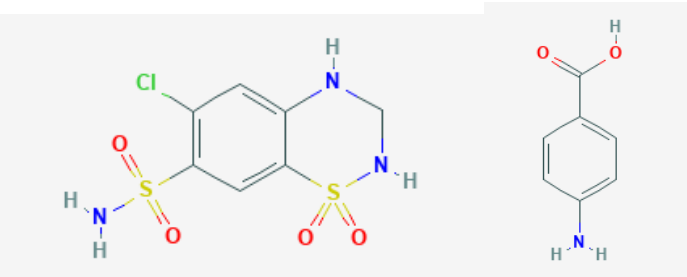


Fig. 1 a. Hydrochlorothiazide, b. Para aminobenzoic acid

defined stoichiometry (Kuminek, Cao, Rocha, Cardoso, & Rodríguez-Hornedo, 2016), which is solid at ambient temperature, with the intermolecular interactions in the unique crystal lattice resulting in weak forces (non-covalent and non-ionic), such as hydrogen bonds, van der Waals bonds (Karagianni, Malamatari, & Kachrimanis, 2018); (Cao, Amidon, Rodríguez-Hornedo, & Amidon, 2018).

The cocrystal design can be done using a supramolecular heterosynthon approach in the presence of functional groups in API molecules and different co-formers. Supramolecular heterosynthons can be formed from carboxylic acids - amides, carboxylic acids - aromatic nitrogen, alcohols-aromatic nitrogen, and alcohol-amines (Duggirala, Perry, Almarsson, & Zaworotko, 2016). The formation of cocrystals of hydrochlorothiazide can carried out by using para-aminobenzoic acid as a conformer, because chemically para-aminobenzoic acid has a carboxyl and amine group that can interact trough hydrogen bonds with the primary and secondary sulphonamide groups of hydrochlorothiazide.

Various methods of cocrystal formation have been reported, and they are divided into solution-based co-crystallization and solid-based co-crystallization (Guo, Sun, Chen, & Cai, 2021). Co-crystallization with solvent evaporation technique is one of the applications of solution-based co-crystallization. In this method, the cocrystal components provided in suitable stoichiometric ratios are dissolved together in a suitable solvent and then allowed to evaporate the solvent completely (Weyna, Shattock, Vishweshwar, & Zaworotko, 2009) (Winantari, Setyawan, Siswodihardjo, & Soewandhi, 2017) .

The objective of this study was to explore co-crystallization of hydrochlorothiazide–para-aminobenzoic acid by solvent evaporation technique. Cocrystals were made using methanol as solvent and allowed at room temperature to evaporate the solvent completely. Cocrystal and their constituent component were characterized by X-Ray Diffraction (XRD) and Differential scanning calorimetry (DSC) instruments. The formation of hydrogen bonds was observed with an FTIR spectrophotometer to determine the intensity changes of the peaks spectra of the functional groups involved in hydrogen bonds formation. Cocrystal morphology

observations were carried out using scanning electron microscope (SEM).

MATERIAL AND METHODS

Chemicals and Instrument

Hydrochlorothiazide were purchased from Changzou Pharmaceutical Factory, Para Aminobenzoic acid were purchased from Sigma Aldrich. Methanol (p.a) from Merck.

The Instruments use were analytical balance (Kern), Differential Scanning Calorimetry/DSC (Perkin Elmer DSC 4000), Fourier Transform Infrared/FT-IR (SHIMADZU IR Prestige), Scanning Electron Microscope/SEM (BRUKER), magnetic stirrer (Heidolph), X-Ray Difractometry/XRD (RIGAKU Mini FlexII).

Co-crystal Formation

Hydrochlorothiazide-para-aminobenzoic acid co-crystals were prepared in molar ratios of 1:0, 1:1, 1:2 and 2:1. Co-crystallization was carried out by solvent evaporation method (Kothur, Swetha, & Bondili, 2012). Hydrochlorothiazide and Para-aminobenzoic acid were put into a beaker and 50 mL of methanol added. The mixture was stirred with a magnetic stirrer at 60°C of temperature and 100 rpm for 1 hour, then transferred to an evaporating dish and allowed to stand at room temperature until the solvent evaporated completely. The cocrystals obtained were collected for further testing.

Characterization of Co-crystal

Scanning Electron Microscope (SEM)

Microscopic observations of the morphology of the raw material and cocrystals of hydrochlorothiazide – para-aminobenzoic acid was carried out by). A small number of samples were placed in a sample holder made of aluminum and coated with gold palladium (Au) with a thickness of 10 nm. The sample then observed at various magnifications. Voltages were set at 10.15 and 20 kV and current to 0.4 mA using Det.BSE and SE.

X-Ray Diffraction

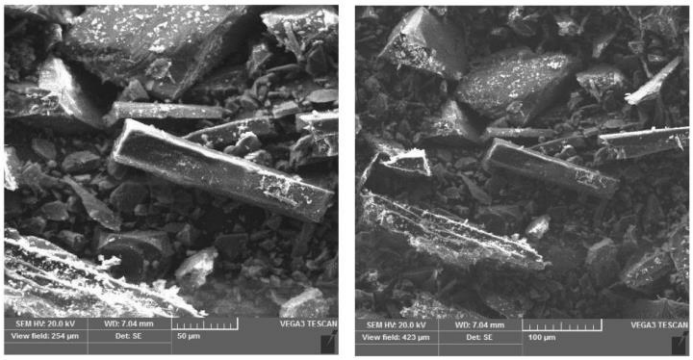
X-ray diffraction patterns of hydrochlorothiazide and cocrystals were carried out using the X-Ray Difractometry (XRD) system with Cu as the stain material and graphite monochromator, operated at a voltage of 30 kV, current 15 mA. Scanning is carried out over an angle range of 10-70° and scanning speed of 4° per minute.

Differential Scanning Calorimetry

DSC analysis of hydrochlorothiazide and cocrystals was carried out using the DSC Perkin Elmer 4000. The samples (10-20 mg) were put into a crucible, with nitrogen as pure gas and flow rate of 20 ml/min. Heating at 30-400°C with a heating rate of 20°C per minute.

Fourier Transform Infrared Spectroscopy

Infrared spectra of hydrochlorothiazide and cocrystals were obtained by means of Fourier Transform Infra-Red (FTIR) Spectrophotometry. Sample preparation was



a b

Fig 2. Cocrystal hydrochlorothiazide – paraminobenzoic acid (1:2) with magnification a). 500x and b) 200x

carried out by pellet technique using the KBr reflectance diffusion method (sample concentration of 2 mg in 20 mg KBr). The measurement was recorded over range 4500-350 cm^{-1}.

RESULTS AND DISCUSSION

Scanning electron microscope (SEM)

Each crystalline material has a hidden atomic structure, which on X-ray irradiation causes constructive and destructive interference of the scattered X-ray beams, resulting in a unique diffraction pattern that presents several sharp points, known as Bragg diffraction peaks (Kaliva & Vamvakaki, 2020). The X-ray diffraction peak is produced by the constructive interference of a monochromatic X-ray beam scattered at a certain angle from each set of lattice planes in the sample. The peak intensity is determined by the distribution of the atoms in the lattice. Consequently, the X-ray diffraction pattern is a fingerprint of the periodic arrangement of atoms in a given material (Bunaciu, Udriştioiu, & Aboul-Enein, 2015).

SEM is used to analyze the shape of cocrystals that are formed with a stronger magnification than optical microscopes so that they can be observed down to the size of a micrometer. Observations using SEM provide visual information about the formation of cocrystals compared to their constituent materials. Based on the figure 2, the 1:2 ratio cocrystals are shaped like long blocks and large and small chunks with varying shapes and sizes. Different crystalline phases of solid particles usually exhibit different crystal habits (Zaini et al., 2020). Crystal habit can affect bulk particle properties such as powder flow properties, bulk density, and cocrystal compressibility (Rasenack et al., 2002).

X-Ray Diffraction (XRD)

Hydrochlorothiazide-para-amino benzoic acid cocrystals molar ratio 1:2 were also analyzed for their diffraction properties using X-Ray Diffraction (XRD). In the 1:2 ratio cocrystal diffractogram (see Figure 3.b), the diffractogram peaks appear at 2ɵ at about 14.904^o; 15.41^o; 20,451^o; 25.553^o; 29.844^o;

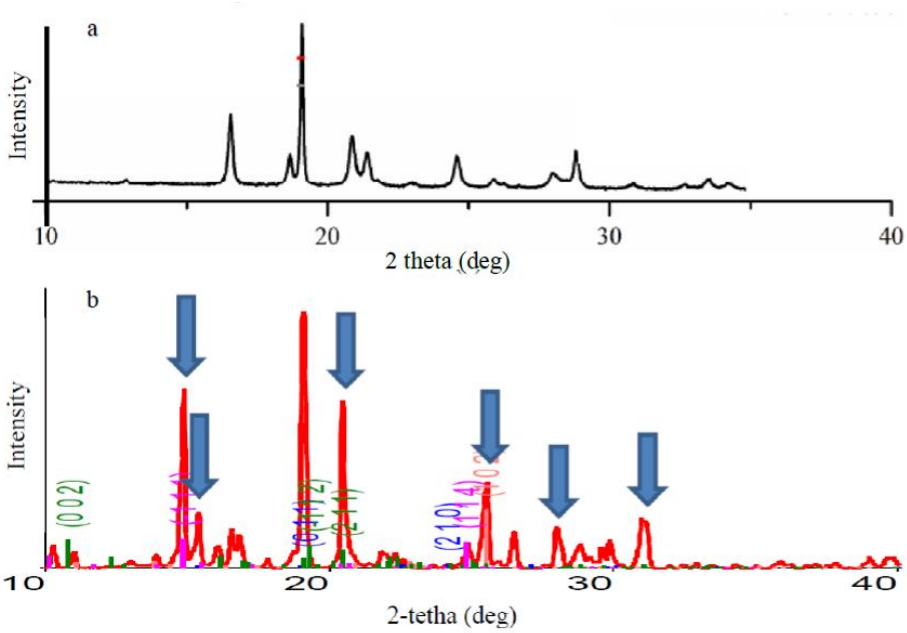


Fig 3. XRD Diffractogram of a) Hydrochlorothiazide, b) Cocrystal 1:2

31.083°. The presence of these new peaks, especially the most obvious at 14.904°, indicates that a new crystalline phase (cocrystal) is formed (Panzade1, Shendarkar, Shaikh, & Rathi, 2017) because it does not appear on the diffractogram of the initial compound. From these results it can be stated that a new crystal is formed because of the interaction between hydrochlorothiazide and para-aminobenzoic acid.

Differential Scanning Calorimetry

The DSC instrument is used to observe and determine the thermal phenomenon of the sample due to temperature changes (Gill, Moghadam, & Ranjbar, 2010). The DSC method can be used as a screening tool to detect cocrystal formation in binary physical mixtures of drugs and their co-formers. This evidence assume that the melting point of the cocrystal is different from its constituent components (Schultheiss & Newman, 2009); (Saganowska & Wesolowski, 2018). Based on the DSC thermogram in Figure 4.a, an endothermic peak of hydrochlorothiazide was produced at 277.17°C and an exothermic peak at 315.67°C, where this endothermic peak indicated the melting point of hydrochlorothiazide while the appearance of an exothermic peak on the thermogram indicated that hydrochlorothiazide was decomposed. While the para-aminobenzoic acid thermogram (Figure 4.b) produced 2 endothermic peaks at 196.43°C and 254.41°C. Based on the shape of the curve for para-aminobenzoic acid, the second endothermic peak at 254.41°C does not indicate the melting point but the presence of the curve indicates that the para-aminobenzoic acid undergoes decomposition. However, it can be concluded that para-aminobenzoic acid has a melting point of 196.43°C.

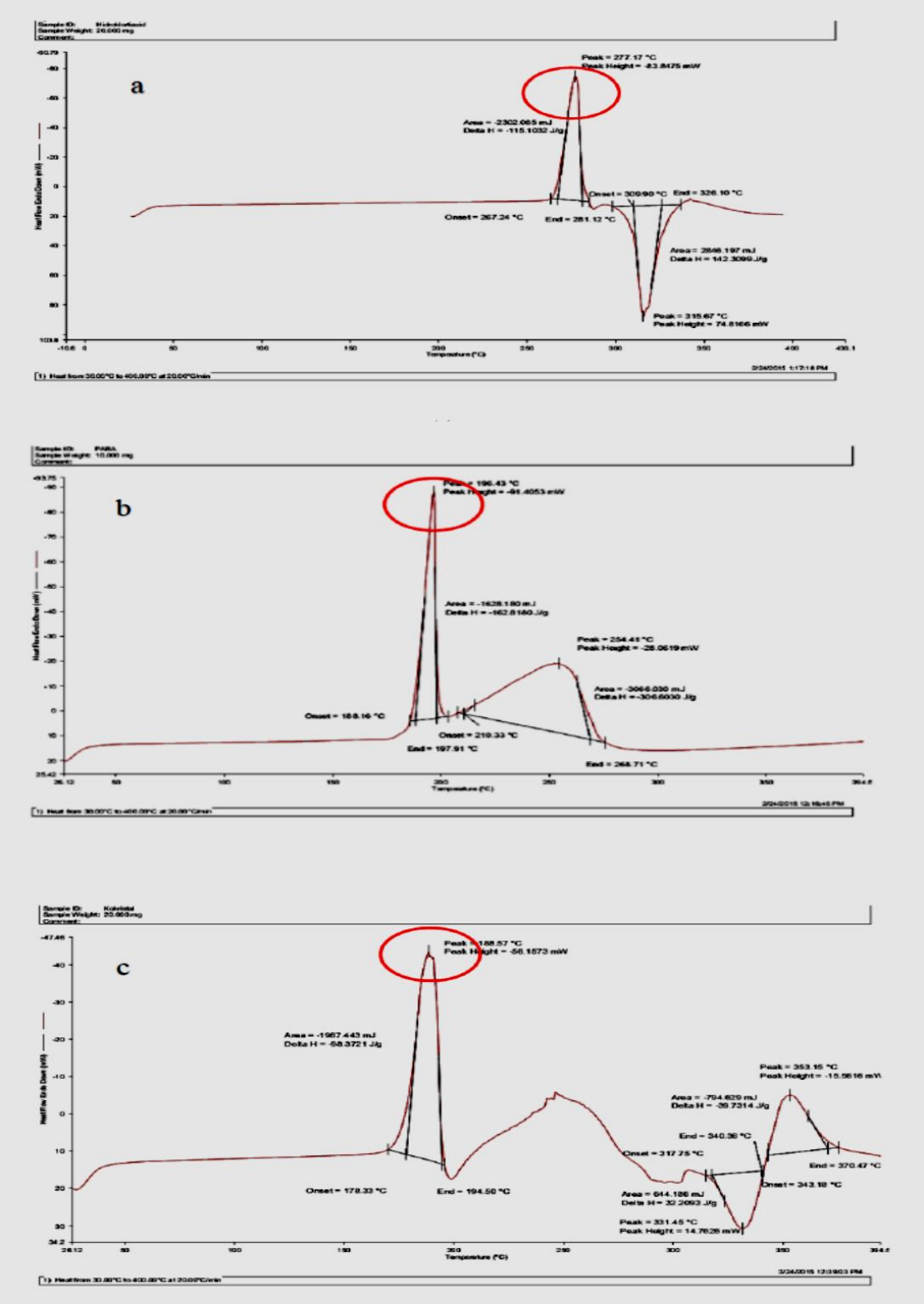


Fig 4. DSC Thermogram of a) hydrochlorothiazide, b) para aminobenzoic acid, c) cocrystal (1:2)

Furthermore, the 1:2 ratio cocrystal thermogram (see Figure 4.c) shows 2 endothermic peaks at 188.57^{o}C and 353^{o}C and one exothermic peak at 331^{o}C. The endothermic peak correlates to the melting of the material (Steinmann et al., 2016), and the sharpest endothermic peak of cocrystal at 188.57^{o}C, which indicates the melting point of cocrystal. The exothermic peak indicates a change in the structure of the compound

which causes a decrease in enthalpy (Leyva-Porras et al., 2020). The DSC profile indicates the occurrence of two main exothermic peaks, which represent the main components of cocrystal (Górska et al., 2020). The mixture of hydrochlorothiazide compounds with para-aminobenzoic acid which has different melting points decreased the melting point temperature to 188.57^{o}C which indicates an interaction between the two compounds and the formation of a new phase. In accordance with Shanpui and Rajput (Sanphui & Rajput, Tuning solubility and stability of hydrochlorothiazide Co-crystals, 2013) that the typical thermogram for hydrochlorothiazide cocrystals with para-amino benzoic acid (1:2 solvent drop grinding method) is typical at the endothermic peak of 175.9^{o}C.

Fourier Transform Infrared Spectroscopy

Based on the results of the infrared spectrum, it can be seen that there is an interaction between hydrochlorothiazide and para-aminobenzoic acid which is indicated by a shift in the infrared wave number in the cocrystal spectrum with a ratio of 1:2. In the spectrum of hydrochlorothiazide (see Figure 5.a), a sharp absorption band with strong intensity at 3361.93 cm^{-1} and 3265.48 cm^{-1} indicates the presence of N-H (stretching). These two absorption bands in the cocrystal spectrum with a 1:2 ratio (see Figure 5.c) shifted to a higher wave number, namely at 3410.15 cm^{-1} and 3356.14 cm^{-1}. The shift in the wave number to a higher wave number indicates a hydrogen bond is formed (Sitorus, 2009).

Absorption band shifts also occur in the sulfonamide groups. The sulfonamide group is a triatomic molecule, in the infrared spectra there is always adjoining absorption for the frequency of symmetry and asymmetry (Sitorus, 2009). Wave number 1373.32 cm^{-1} (asymmetric secondary sulfonamide) and 1319.31 cm^{-1} (asymmetric primary sulfonamide) shifted to 1379.10 cm-^{1} accompanied by an increase in absorption intensity and 1321.24 cm^{-1}. The frequency of the symmetrical sulfonamide groups also shifted from 1151.50 cm^{-1} to 1172.72 cm^{-1}.

Furthermore, the para-aminobenzoic acid spectrum (see Figure 5.b) shows a sharp absorption band with medium intensity at of 3230.77cm^{-1} experiencing a higher shift to 3257.77 cm^{-1} at a cocrystal spectrum with a ratio of 1:2. The shift indicates an O-H shift (stretching). The absorption band shift also occurs in the N-H (stretching) spectrum of para-aminobenzoic acid from wave number 3361.93 cm^{-1} to wave number 3410.15 cm^{-1} in the cocrystal spectrum ratio 1:2. The absorption band shift also occurs at C=O (stretching) in the para-aminobenzoic acid spectrum which shifts from 1662.64 cm^{-1} to 1685.79 cm^{-1} in a cocrystal spectrum.

The shifting of absorption peaks of FTIR spectra of hydrochlorothiazide-para-aminobenzoic acid cocrystal indicated appearance intermolecular hydrogen bond

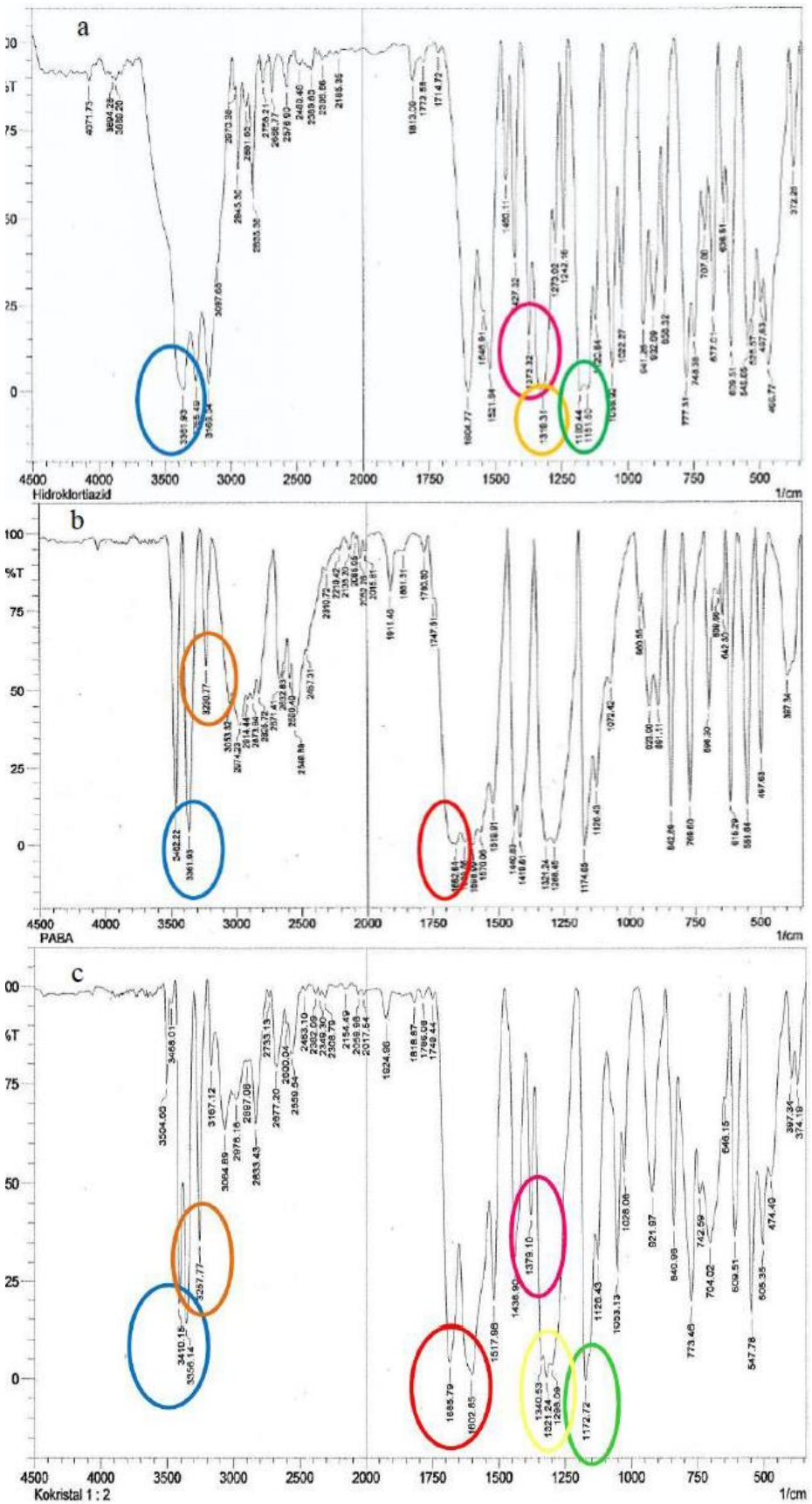


Fig 5. FTIR spectra for a. Hydrochlorothiazide, b. para aminobenzoic acid; c. cocrystal (1:2)

interactions between functional groups of hydrochlorothiazide and para-aminobenzoic acid (Wicaksono et al., 2019). Cocrystal results from the interaction of hydrogen

bonds between the amine group of hydrochlorothiazide and the carboxylic group of para-amino benzoic acid, as indicated by the chemical shift of the sulfonamide groups from 1151.50 cm^{-1} to 1172.72 cm^{-1} and band shift of C=O (stretching) in the para-aminobenzoic acid from 1662.64 cm^{-1} to 1685.79 cm^{-1} in a cocrystal.

CONCLUSION

The hydrochlorothiazide-para-aminobenzoic acid cocrystal has new crystalline peaks at 2θ of 14.904º; 15.41º; 25.553º; 26.5º; 29.844º; 31.083º indicating the formation of a new crystalline phase. The cocrystal showed the melting point at 188.57ºC which is different from the initial components. The FTIR spectra of cocrystal showed the shifting of absorption peaks of groups of initial components indicating of formation of hydrochlorothiazide-para-aminobenzoic acid cocrystal through intermolecular hydrogen bond interactions between amine/sulfonamide group and carboxyl group.

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