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Study of the chemical compatibility of two active substances and stability of their solution

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ABSTRACT

The metabolic field aiming at theoretical and applied analysis of metabolic processes of different levels to be the basis or the background of many diseases has been actively developing for the last decade. Especially, the concepts of the role of the cell energy metabolic imbalance during various pathologic processes that are based, first of all, on the mitochondrial deficiency have been actively developed.The irreversible cell damage when affected by the disturbing agents occurs from the moment of damage of organelles- the ATP generating cell systems. The paper studies the opportunity of creating the combined dosage formulation containing two active pharmaceutical ingredients (succinic acid and butafosfan) based on their chemical compatibility. Butafosfan improves the blood glucose disposal that stimulates the energy metabolism and accelerates metabolic processes due to the ADP-ATP cycle stimulation. Succinic acid can directly improve the energy-synthesizing function of mitochondria by increasing the succinate delivery and consumption by ischemic cells, participate in implementation of the succinic acid quick oxidation by succinate dehydrogenase, as well as activation of the mitochondrial respiratory chain leading to the ATP quick resynthesis. One of the obligatory phases of research is identification and study of physical and chemical properties of the active ingredient, compatibility assessment of the active ingredient and excipients, and in case of combination drugs - also compatibility assessment of ingredients themselves. To choose the necessary conditions during the solution technology development, the chemical properties, stability and compatibility of these pharmaceutical substances were studied.

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INTRODUCTION

The modern treatment any pathology includes a combination of two or more drugs in the well-tolerated doses. The use of combination medicines strengthens organoprotection, since there is a complementary effect on the target organs due to the physiological and pharmacological synergy between products of different classes (Hisao and Masuo, 1989; Brabander *et al.*, 2009). The main task of any pharmaceutical development is to produce a qualitative, effective and safe product. That is why one of the obligatory phases of research is identifica-

tion and study of physical and chemical properties of the active ingredient, compatibility assessment of the active ingredient and excipients, and in case of combination drugs - also compatibility assessment of ingredients themselves (Viñas *et al.*, 2004).

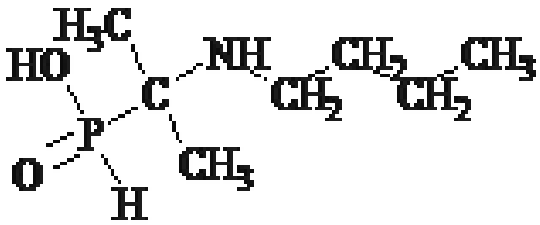

Figure 1: Butafosfan structural formula

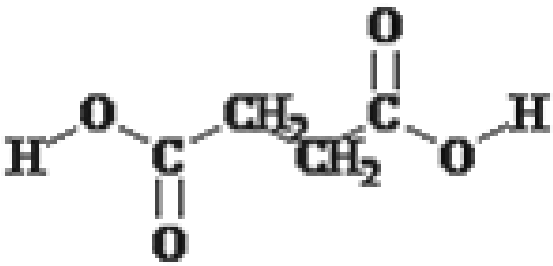

Figure 2: Succinic acid structural formula

The objective of this work is to study the chemical compatibility of two pharmaceutical substances in the same dosage formulation and with excipients. The developed dosage formulation is a solution containing two active pharmaceutical ingredients: succinic acid and butafosfan (Zai *et al.*, 2013; Singh, 2015; Cristina *et al.*, 2012).

MATERIALS AND METHODS

The main phases in research of the chemical compatibility of succinic acid and butafosfan in the same dosage formulation were: study of the chemical compatibility of two substances (both jointly and together with excipients) in the form of model solutions.

Butafosfan (1-butylamino-1-methyl) is a phosphorus organic compound. Chemical name - ethyl phosphonic acid (Figure 1). By its pharmacological properties, butafosfan belongs to the metabolic process stimulants in the organism.

Succinic acid (butanedioic acid, ethan-1,2-dicarboxylic acid) is the dicarboxylic acid (Figure 2) consisting of four carbon atoms and occurring in the vegetable and animal tissues. In the drugs, succinic acid is used as active ingredient being a metabolic product improving the tissue metabolism and energy support reducing the tissue hypoxia (Udalova *et al.*, 2015) .

The first experimental phase in compatibility research of the combination drug consisted in monitoring the creation of the identified solutions of the test substances and, perhaps, new (non-identified) substances when occurring in the combination dosage formulation (Singh, 2015).

Identification of chemical substances in the solutions and the mixture of two ingredients was performed by mass-spectrometry with ionization using the electrospray (ESI-MS-analysis) at the Amazon X device (Bruker Daltonik GmbH, Germany). The nitrogen with the temperature of 220 ^{0}C is used in the source as the drying gas. The voltage in the source was 4.5 kV. The sample was introduced using the liquid chromatograph auto-sampler Agilent 1260 Infinity (Agilent Technologies, USA). Isopropanol eluent: water, 50:50, eluent flow rate 0.2 ml/min. The spectra were recorded in the record mode of the negatively charged particles (Carrasco *et al.*, 2008; Mahmoudi *et al.*, 2014; Chilumuru *et al.*, 2015).

RESULTS AND DISCUSSION

The mother solutions of butafosfan and succinic acid were prepared in the de-ionized water, with 1 mg/ml concentration. The samples were very soluble, without sedimentation. Then the solutions were centrifuged for 5 min. at 14 ths. rpm for trace impurity and solid particle deposition. The mother solutions were diluted before spectra recording to the 20 μg/ml concentration. The pure spectra of butafosfan and succinic acid were obtained in the record mode of the negatively charged particles.

In the mass-spectrum ESI-MS (Figure 3), the peaks m/z 178 and 357 belong to the ions $[M-H]^-$ and $[2M-H]^-$ butafosfan. The high intensity, peaks m/z 178, makes it preferable when analyzing this substance in different mixtures.

In the mass-spectrum ESI-MS (Figure 4), the peaks m/z 117 and 235 belong to the ions $[M-H]^-$ and $[2M-H]^-$ Consequently, these peaks are analytical when determining the succinic acid. The high intensity, peaks m/z 117, makes it preferable when analyzing this substance in different mixtures.

Further on, to define the chemical compatibility, the mixture of mother solutions of 10% butafosfan and 1% succinic acid was prepared in 1 mL of de-ionized water. The mixture was mixed in the shaker for 1 min. and then centrifuged for 5 min. at 14 ths. rpm. Then, the pure spectrum of the butafosfan and suc-

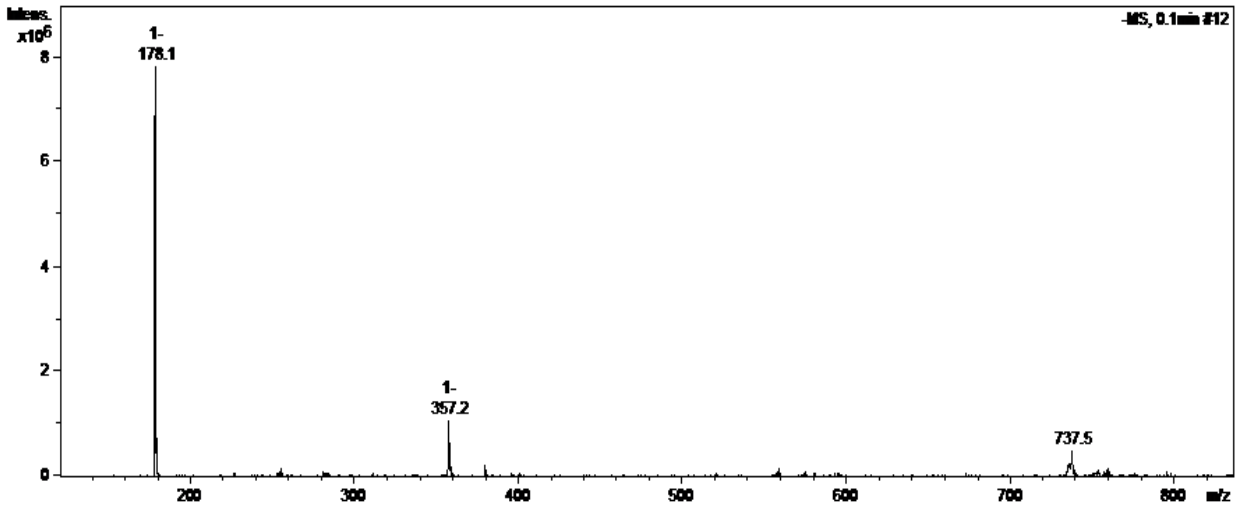

Figure 3: ESI-MS analysis of butafosfan solution

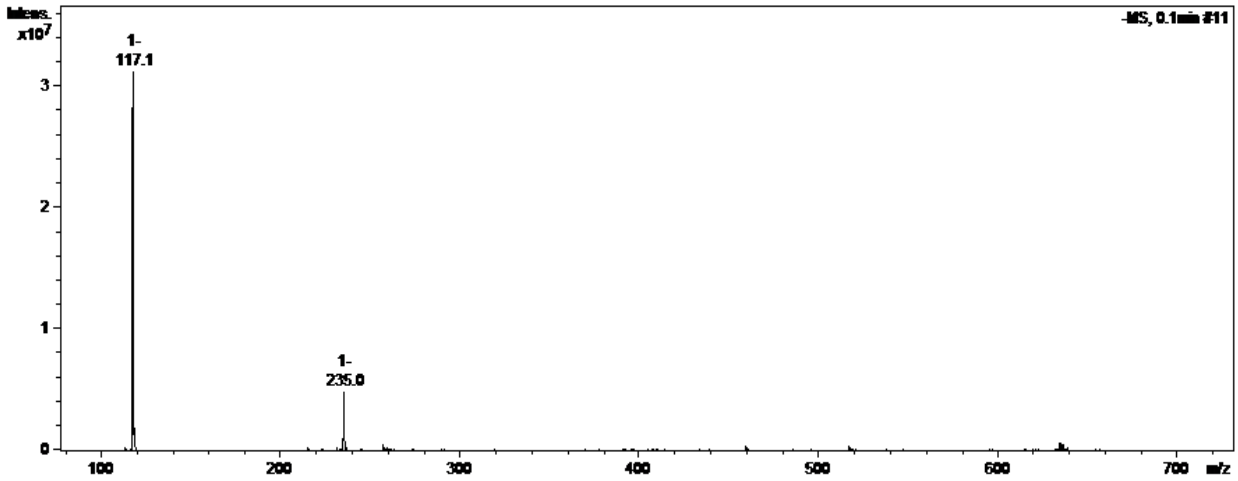

Figure 4: ESI-MS analysis of succinic acid solution

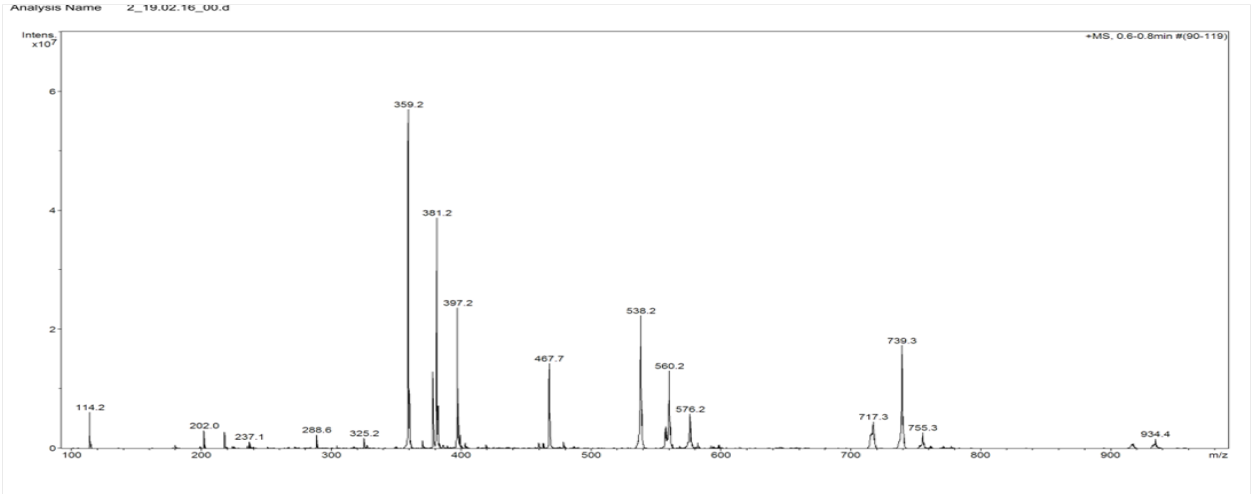

Figure 5: ESI-MS analysis of the mixture of the 10 % butafosfan and 1% succinic acid, 0.5 % novocaine solutions

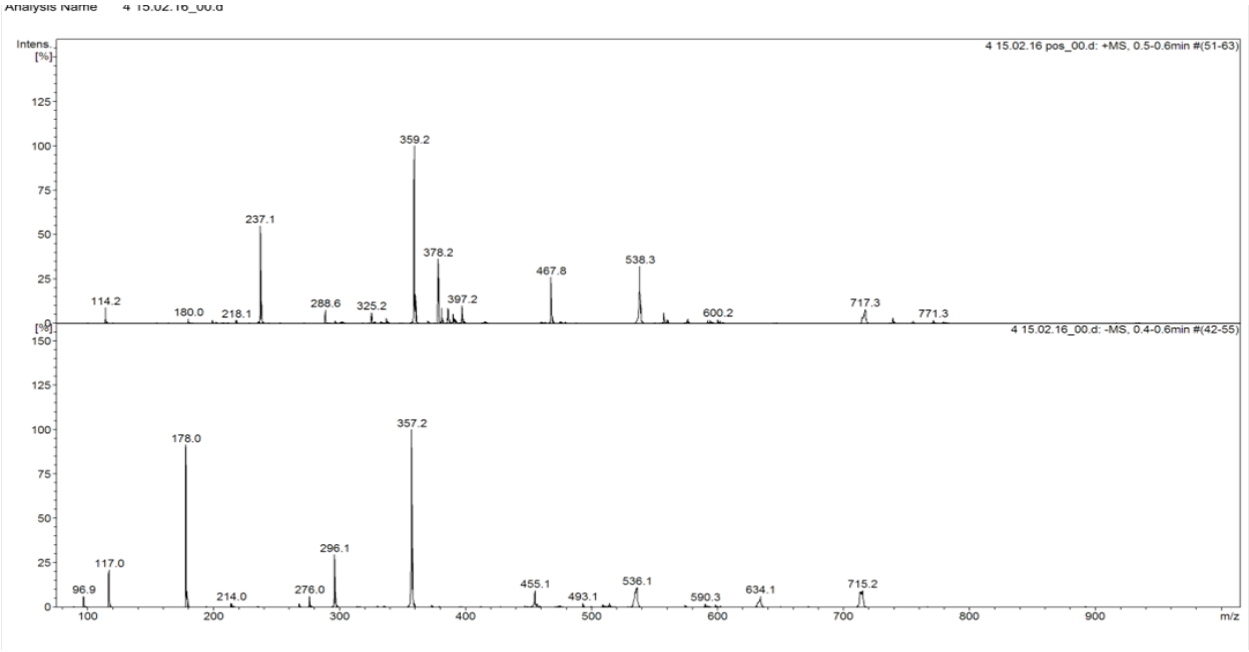

Figure 6: ESI-MS analysis of the mixture of the 10 % butafosfan and 1% succinic acid, 0.5 % novocaine solutions in 6 and 12 months of storage period

cinic acid mixture was obtained.

The analysis of the mass-spectrum of the mixture of butafosfan and succinic acid and novocaine (Figure 5) indicates that no interaction between the mixture components occur. There are no peaks with the other values m/z in the mass-spectrum. The ion peaks m/z 117 and m/z 178 belong to the analytical peaks of the succinic acid and butafosfan molecules, respectively. The intensity ratio of these peaks reflects their concentration in this mixture.

The 0.5% novocaine solution was prepared in the de-ionized water in a similar way. In the mass-spectrum of this specimen ESI-MS, the peak m/z 235 of ion $[M-H]^-$ novocaine was not observed in the mode of the negatively charged particles. In view of this, the identification method of novocaine by the mass-spectra ESI-MS in the mode of the positively charged particles was used. In this case, the intense peak m/z 237 of ion $[M+H]^+$ was observed in the mass-spectrum. Consequently, this peak is analytical when determining the novocaine in the samples.

Creation of the medicinal product assumes the determination of shelf life and substantiation of the optimal storage periods and conditions. The properties of the medicinal product should be determined and standardized as per pharmacopoeial requirements. For standardization and quality control of the complex antibacterial products, as well as for stability study during storage, the assessment methods of the medicinal product applied to the injection solutions were used including the determination of the appearance and concentration of the active ingredients in the solutions. The shelf life was established according to the stability study results in the long-term storage conditions of the solutions at +5±2 °C for 6 and 12 months in the light-protected area. The organoleptic control of the model solutions showed that all the mixtures withstood the requirements to the appearance during the shelf life of 6 and 12 months, that is: the solutions are transparent, without impurities.

During identification of substances of the model mixtures when stored in the mass-spectrum in the mode of the negatively charged particles of the sample (12 months for the 10 % butafosfan and 1% succinic acid, 0.5 % novocaine solutions) (Figure 6), the main components - succinic acid and butafosfan - are clearly visible. Peaks with other values m/z are minor. The ion peaks m/z 117 and m/z 178 mentioned above belong to the analytical peaks of the succinic acid and butafosfan molecules, respectively. The presence of novocaine in this sample is established by the mass-spectrum in the mode of the positively charged particles. The intense peak m/z 237 of ion $[M+H]^+$ was observed in the mass-spectrum of this specimen.

CONCLUSIONS

Therefore, using the mass-spectrum method in the modes of the negatively and positively charged particles, identification of butafosfan, succinic acid and novocaine was performed. The intensity ratio of

these peaks reflects their concentration in this mixture, thus evidencing the stability of the produced mixture in 12 months of the storage period in the light-protected area at +5±2 °C.

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Conflict of Interest

None.

REFERENCES

Brabander, H. F. D., Noppe, H., Verheyden, K., Bussche, J. V., Wille, K., Okerman, L., Vanhaecke, L., Reybroeck, W., Ooghe, S., Croubels, S. 2009. Residue analysis: Future trends from a historical perspective. *Journal of Chromatography A*, 1216(46):7964–7976.

Carrasco, P. A., Casado, T. S., Segura, C. A., Fernández, G. A. 2008. A reversed-phase high-performance liquid chromatography coupled to ultraviolet and electrospray time-of-flight mass spectrometry online detection for the separation of eight tetracyclines in honey samples. *J Chromatogr A*, 9:107–116.

Chilumuru, R. M., Lakkineni, C. A., Chandra, B. S. 2015. Quantitative Analysis of Oxytetracycline Residues in Honey by High Performance Liquid Chromatography. *J. Biological Sci*, 4(5):59–65.

Cristina, C., Gabriela-Alina, D., Mirel, G., Delia, P. 2012. Stability of tetracycline residues in honey. *Journal of the Serbian Chemical Society*, 77(7):879–886.

Hisao, O., Masuo, Y. 1989. Improvement of chemical analysis of antibiotics. *J. Chromatography*, 6:315–322.

Mahmoudi, R., Moosavy, M., Norian, R., Kazemi, S., Nadari, M., Mardani, K. 2014. Detection of Oxytetracycline Residues in Honey Samples Using ELISA and HPLC. *Methods. Pharmaceutical Sciences*, 19(4):145–50.

Singh, S. P. 2015. Validation of an analytical methodology for determination of tetracyclines residues in honey by UPLC - MS/MS detection. *Indian Journal of Natural Products and Resources*, 6(4):293–298.

Udalova, A. Y., Dmitrienko, S. G., Apyari, V. V. 2015. Methods for the separation, preconcentration, and determination of tetracycline antibiotics. *Journal of Analytical Chemistry*, 70(6):661–676.

Viñas, P., Balsalobre, N., López-Erroz, C., Hernández-Córdoba, M. 2004. Liquid chromatography with ultraviolet absorbance detection for the analysis of tetracycline residues in honey. *Journal of Chromatography A*, 1022(1-2):125–129.

Zai, M., Rehman, K., Hussain, A. 2013. Detection and Quantification of Antibiotics Residues in Honey Samples by Chromatographic Techniques. *+J.+Scientific+Research*, 14(5):5–5.