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Using Raman Spectroscopy to Investigate Drugs with Similar Chemical Structures and Antagonistic Effects

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SUMMARY.This paper presents a Raman spectroscopy analysis of drugs with similar chemical structures and antagonistic effects. The research objects selected for analysis were folic acid, which has a vitamin effect, and its antagonist methotrexate; sulfanilamide derivative – streptocide; and p-aminobenzoic acid derivative – benzocaine. It was demonstrated that these drugs, which are identical in terms of physical and chemical properties, can be identified and distinguished from each other using the Raman spectroscopy method.

RESUMEN. Este artículo presenta un análisis de espectroscopía Raman de fármacos con estructuras químicas similares y efectos antagónicos. Los objetos de investigación seleccionados para el análisis fueron el ácido fólico, que tiene efecto vitamínico, y su antagonista metotrexato; derivado de sulfanilamida – estreptocida; y derivado del ácido p-aminobenzoico – benzocaína. Se demostró que estos fármacos, idénticos en cuanto a propiedades físicas y químicas, pueden identificarse y distinguirse entre sí mediante el método de espectroscopia Raman.

INTRODUCTION

It is well established that the pharmacological properties of drugs are directly related to their chemical structure. Despite the chemical similarity of some drugs, they can exhibit antagonistic pharmacological effects. Folic acid and methotrexate belong to the group of vitamins B and are classified as pteridine derivatives. Folic acid is a chemically complex substance consisting of 2-amino-4-oxy-pteridine, p-aminobenzoic acid and glutamic acid. Methotrexate differs from folic acid in that its pterin ring has an amino group instead of a hydroxyl group in position 4 and a methyl group instead of a hydrogen in position 10. Folic acid, also known as vitamin B9, plays a pivotal role in the biochemical processes that occur within a living organism. A deficiency of folic acid can lead to a disruption of the hematopoietic function of the body, ultimately resulting in the development of anaemia [1] (Fig. 1).

It can be observed that methotrexate, derived from the chemical structure of folic acid, has not only ceased to exhibit the properties of a vitamin, but also exerts an adverse effect on folic acid. Furthermore, it can be established that methotrexate has a stronger anti-vitamin effect than that of folic acid. The drug is employed as a therapeutic agent in gynecological practice, in the treatment of rheumatoid arthritis, acute leukemia, lung and breast cancer [2].

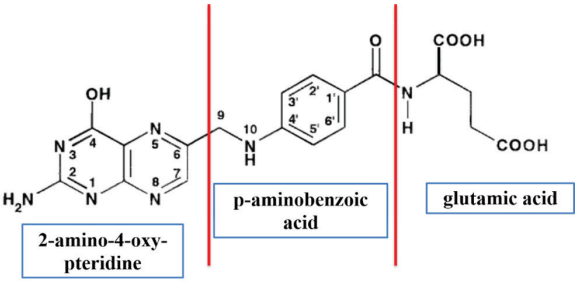

Figure 1. Chemical structure of folic acid.

KEY WORDS: benzocaine; folic acid; methotrexate; Raman spectroscopy; streptocide.

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Both drugs exhibit analogous physico-chemical properties, with the former described as an orange-yellow crystalline powder that is slightly soluble in alkalis and insoluble in water and ethanol. Furthermore, the angle of polarization of folic acid and methotrexate is almost identical, at +19 - +22º (1% solution in 0.1 M NaOH) and +19 - +24º (1% solution in Na_2CO_3), respectively.

In accordance with the normative document, the authenticity of folic acid can be ascertained through the oxidation of the compound, in the presence of potassium permanganate, the disintegration of folic acid into its constituent elements, and the observation of a blue fluorescence upon irradiation with ultraviolet (UV) light of a wavelength of 254 nm. This fluorescence of the drug is due to the pteric acid that is formed as a result of the oxidation of the drug. Methotrexate is also oxidised in the presence of potassium permanganate and gives a blue fluorescence.

In addition, a 0.001% solution of folic acid in 0.1 M NaOH absorbs the maximum light at wavelengths of 256, 283, and 365 nm when folic acid is determined spectrophotometric method according to the State Pharmacopoeia. A solution of an equal concentration in the same solvent absorbs light at 258, 303 and 370 nm when methotrexate is analysed by this method.

According to the normative document of folic acid, the TLC method is also introduced, using organic solvents such as ammonia solution, propan-1-ol, ethanol as mobile phase and methanol and ammonia solution for sample preparation.

Other drugs of this type have similar chemical structures but opposite pharmacological effects, it is possible to mention sulfanilamide derivative - streptocide, p-aminobenzoic acid derivative - benzocaine.

The bacteriostatic effect of sulphanilamide drugs is based on the fact that they interfere with the process of forming folate, hydrofolic acid and other substances that contain p-aminobenzoic acid residues in their molecular structure, which are necessary for bacteria to live in the body. Folic and hydrofolic acids are formed from p-aminobenzoic, glutamic acids, pterin and hydropterin.

In the biosynthesis of folic and hydrofolic acid, sulfanilamide, instead of p-aminobenzoic acid, is ingested by the body and alters its chemical structure, creating a compound that is not suitable for the survival and reproduction of microorganisms. Despite the structural similarities and certain properties, between sulfanilamide drugs and p-aminobenzoic acid, the exchange process is disrupted when microbial cells accept sulfanilamide drugs. This suggests a potential incompatibility between sulfonamides and p-aminobenzoic acid.

Therefore, in order to determine the authenticity of this group, it is essential to use analytical techniques that are specific to the entire molecule, rather than relying on the analysis of individual functional groups.

The objective of this research is to investigate of drugs that are similar in chemical structure but opposite in terms of pharmacological effects, using the Raman spectroscopy method, allows for the determination of intermolecular bonds and polymorph – enantiomers.

Folic acid, which has the effect of a vitamin, and its antagonist, methotrexate, as well as the sulphanilamide derivative, streptocide, and the p-aminobenzoic acid derivative, benzocaine, were selected as research objects. Folic acid and methotrexate were purchased from Pharmaffiliates, India. Benzocaine and streptocide were purchased EDQM.

MATERIALS AND METHODS

The analyses were carried out on the Raman spectrometer of the "Enhanced Spectroscopy" company, model "R-532". Parameters of the device: the spectral range from 100 to 6000 cm^{-1}, spectral resolution of 5-8 cm^{-1}, the entrance slit 20-30 microns, a holographic diffraction grating 1800 lines/mm, a set of highly selective and cut filters, as well as 50 mW single mode laser with a wavelength of 532 nm. The measurement was performed at room temperature.

RESULTS AND DISCUSSION

A Raman spectrum analysis of folic acid reveals the presence of intense combination scattering lines within the 200–1600 and 2800–3000 cm^{-1} regions [3,4]. Analysing the lower spectrum area first, we can see that the deformation vibrations of the C-C bond are located at 399, 523 cm^{-1}, and the valence vibrations of the ν C-C bond are recorded at 638, 679 cm^{-1}. The symmetric valence vibrations of the high-intensity C-N-C bond have a frequency at 846 cm^{-1}, while the aromatic ring C-C vibrations have a frequency at 1037 cm^{-1}, and the vibrations of the p-substituted benzene ring occur with a frequency of 1123 cm^{-1}. Moreover, it can be posited that the C-O asymmetric valence vibrations are recorded at 1237 cm^{-1}, whereas the C-H deformation vibra-

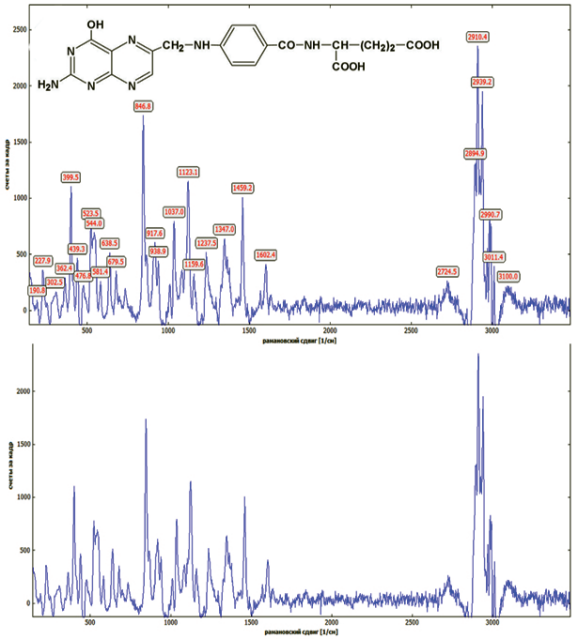

Figure 2. The Raman spectrum of the folic acid substance.

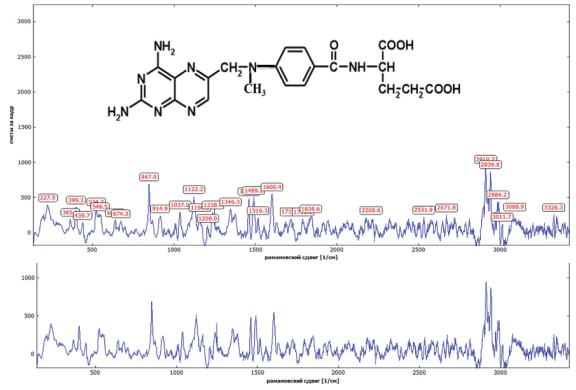

Figure 3. The Raman spectrum of the methotrexate substance.

tions are observed at 1347 cm^{-1}. The aromatic nitrogen present in the pteridine ring is observed at 1459 cm^{-1}. It can be seen that the symmetric and asymmetric valence vibrations of the methylene (CH_2) group are located at 2894 and 2939 cm^{-1}, respectively (Fig. 2).

The Raman spectrum of methotrexate reflects the various chemical characteristics of its structure, including the presence of a -CH_3 radical bonded to the nitrogen at the 10th position of folic acid, as observed at 1489 cm^{-1} [5]. Additionally, the scissor vibrations of the -NH_2 group instead of the -OH group, in the 4th position are present at 1516 cm^{-1} (Figs. 3 and 4).

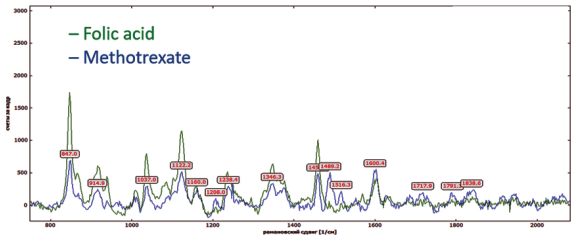

Figure 4. Raman spectra of folic acid and methotrexate.

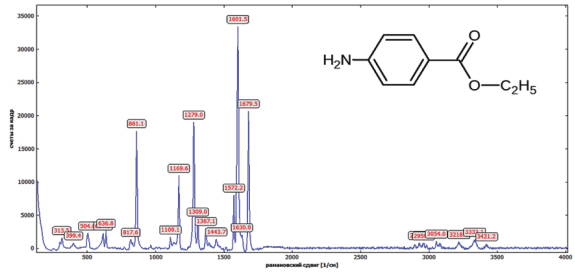

Figure 5. The Raman spectrum of the benzocaine substance.

One of the next antagonist group drugs, benzocaine, was subjected to Raman spectroscopy. Analysis demonstrated the presence of symmetric valence vibrations of the C-O-C ether bond at 861 cm^{-1}, as well as the asymmetric valence vibrations at 1279 cm^{-1}, resulting in the formation of intense combination scattering lines. Additionally, the wagging vibrations of the –NH_2 functional group resulted in the appearance of scattering lines with low intensity at 636 cm^{-1}, while the scissors deformation vibrations exhibited moderate intensity at 1572 cm^{-1}. The bands with the highest intensity correspond to the -C=C- bond in the aromatic ring and the-C=O bond in the carboxyl group, which can be observed at 1601 cm^{-1} and 1679 cm^{-1}, respectively [6,7] (Fig. 5).

In the Raman spectrum of streptocide drugs from sulfanilamides, which have been deemed antagonists of p-aminobenzoic acid due to their pharmacological effects, specific variations in the intensity of certain functional groups, which are distinct from the chemical structure of benzocaine, have been observed (Table 1).

In particular, the scissor vibrations of the OSO group were observed to form scattering lines with an average intensity of 561 cm^{-1}, while the symmetric valence vibrations exhibited the highest intensity at 1133 cm^{-1}. Furthermore, the valence vibrations of C-S, C-N, and S-N bonds

Benzocaine		Streptocide	
Assignment	**Frequencies (cm^{-1})**	**Assignment**	**Frequencies (cm^{-1})**
ν CC	313 $_{w}$	ν CC	296 $_{m}$
ν CC	399 $_{vw}$	ν CC	369 $_{w}$
δ CC	504 $_{w}$	δ CC	508 $_{w}$
	–	δ CC	537 $_{w}$
	–	$(OSO)_{scissoring}$	561 $_{m}$
$-NH_{2\ wagging}$	636 $_{w}$	$-NH_{2\ wagging}$	633 $_{m}$
	–	ν CS	712 $_{m}$
	–	ν CS	820 $_{m}$
	–	(CS) + (CN)	840 $_{s}$
ν COC	861 $_{s}$		–
	–	ν SN	898 $_{m}$
	–	sulfonamide	1092 $_{s}$
	–	ν_s (OSO)	1133 $_{vs}$
p- substituted benzene ring	1169 $_{m}$	p- substituted benzene ring	1155 $_{m}$
ν_{as} COC	1279 $_{s}$		–
δ CH	1309 $_{m}$	δ CH	1313 $_{w}$
δ_s (CH) in CH_3	1367 $_{m}$		–
δ-$NH_{2\ scissoring}$	1572 $_{m}$	δ-$NH_{2\ scissoring}$	1592 $_{vs}$
ν C=C	1601 $_{vs}$	ν C=C	1627 $_{m}$
-C=O	1679 $_{vs}$		–
ν_{as} CH	2959 $_{vw}$		–
$CH_{aromatic}$	3054 $_{vw}$	$CH_{aromatic}$	3067 $_{m}$
ν_s $-NH_2$	3333 $_{vw}$	ν_s $-NH_2$	3373 $_{s}$
ν_{as} $-NH_2$	3421 $_{vw}$	ν_{as} $-NH_2$	3476 $_{m}$

vs = very strong; s = strong; m = medium; w = weak; vw = very weak

Table 1. Frequencies (cm^{-1}) and tentative assignments of the bands in the Raman spectra of benzocaine and streptocide.

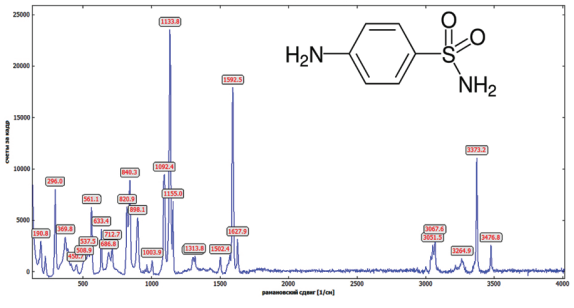

Figure 6. The Raman spectrum of the streptocide substance.

lie within the range of 710 cm^{-1} to 900 cm^{-1}. The streptocide molecule contains a primary aromatic amine, $-NH_2$ (attached directly to the benzene ring), and a sulfonamide group, also $-NH_2$ (attached to sulfur). Their symmetric valence vibrations, with a frequency of 3373 cm^{-1}, and their asymmetric valence vibrations, with a frequency of 3476 cm^{-1}, form combination scattering lines, which have a medium intensity. The scissor deformation vibrations of the $-NH_2$ group, as well as the vibrations of the -C=C- bond, have been observed to shift in frequency by 20 cm^{-1} and 25

cm^{-1}, respectively. The new observed frequencies are 1592 cm^{-1} for the scissor deformation vibrations, and 1627 cm^{-1} for the vibrations of the -C=C- bond [8,9]. The absence of a complex ester bond in the structure of the streptocide molecule precluded the identification of scattering lines related to the carbonyl (C=O) and the carbonate ester (COC) bonds (Fig. 6).

CONCLUSION

The potential application of Raman spectroscopy for the analysis of pteridine derivatives was investigated. In the Raman spectra of folic acid and methotrexate, which have analogous physicochemical properties and chemical structures, demonstrate antagonistic pharmacological effects in contrast to each other. The combination scattering lines characteristic to the functional groups of the drugs were determined, and it was proven that this method could be used to distinguish them from each other. Furthermore, the benzocaine (derivative of p-aminobenzoic acid) and streptocide (sulphanilamide derivative), which are considered antagonistic to it in terms of their pharmacological effect, were also studied using Raman spectroscopy. The specific scattering lines of each were elucidated.

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