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Analysis of Over-the-Counter Antihistamines Through Raman Spectroscopy and Density Functional Theory Calculations

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Abstract

Antihistamines are a class of chemical agents often used in the management of allergies. They treat the nasal, skin, and gastric irritations caused by the body's histamine receptors. These medications can be taken as an oral tablet, nasal spray, eye drop, and injection. In this study, common over-the-counter antihistamine tablets were analyzed by Raman spectroscopy. The aim of the research is to use Raman spectroscopy, as well as density functional (DFT) calculations, to characterize generic antihistamines such as diphenhydramine, loratadine, fexofenadine, chlorpheniramine, and cetirizine. The difference in the functional groups of each allergy drug was analyzed through its vibrational bands, showing both wavelengths common in most antihistamines and unique wavelengths in specific antihistamines. Causes of weak Raman activity in experimentally run pharmaceutical tablets was also discussed.

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Introduction

History of Raman Spectroscopy

Raman spectroscopy is a method of vibrational spectroscopy that is both non-invasive and non-destructive. It provides information about the molecular composition, structure, and interactions within a compound. Raman utilizes the scattering patterns of photons within the molecule. The photons are moved to a different vibrational state than the ground, either higher or lower. The photon gains or loses the same amount of energy as the difference between its ground vibrational state and new state[3]. The use of photons to measure the activity does cause issues of interference with fluorescence in some compounds. This interference is caused by the photon being excited past the normal Raman energy levels into the resonant fluorescence range. The fluorescence is significantly stronger, and dampens any Raman activity that would be represented on the normal spectrum[47]. Raman spectroscopy also relies on induced changes in the polarizability of a molecule, which is the ability of the electrons to move after stimulus from an external electric field. Molecules are polarized in different vibrational modes: symmetrical stretching, asymmetric stretching, and bending. Asymmetric vibrations do not appear in Raman spectra. Bending vibrations appear as weak modes within Raman, and symmetric stretches appear strongly[1].

Raman scattering was first reported in 1928 by physicists K. S. Krishnan and C. V. Raman. C. V. Raman went on to earn the physics Nobel prize in 1930 for his work in the discovery of Raman light scattering. Raman utilized a simple telescope and focused sunlight through the scope onto an organic sample. He then collected the scattered radiation through

optical filters and a secondary lens. After their discovery of the phenomenon, a large gap in development took place, with the next advancement in the 1960s. The original set-up of the spectroscopic instrument left incident light that was significantly more intense than the scattered light, leaving no practical Raman light source[50]. After the development of the laser in 1960, Raman scattering returned to the limelight, as research was done to utilize the focused laser beam and newer optical discoveries in experimental use of the scattering[1]. The late 1970's saw the development of an optical microscope with Raman spectrometer attachment, allowing the analytical method to grow in popularity.

History and Functions of Histamine Receptors

Histamine was first isolated from the mold ergot by Henry Dale in 1910. Following its discovery and isolation, Dale continued to explore histamine's biological actions, the first study on the protein. This study was completed by injecting the ergot histamine into different animals, and observing the results. For many species, the histamine caused severe respiratory and vascular function difficulties, and typically ended in a relatively quick death[51]. This led to further attempts in isolating human histamine, as it was now clear that histamine proteins were not universal. This isolation occurred in 1927, with a successful isolation of histamine from from the liver and lungs[52]. After the isolation of the human histamine protein, more research was conducted to determine its functions and pathways.

It was ultimately determined that histamine is primarily stored in mast cells. Mast cells are seen in virtually all human tissues, and are attributed to inflammation within the body. The cells are often found in high quantities in the nasal epithelium of peoples suffering from allergies. This increased quantity lead to the realization of the role of histamines in allergies.

When histamine is first released from mast cells, it triggers the early phase of allergic reactions, whether moderate or severe. The histamine molecules bind to the appropriate H_1- or H_2-receptors, causing the nerve activity that leads to the physical symptoms of an allergic reaction[53].

History of Antihistamines

Antihistamines were first recognized for their anti-allergy effects when utilized in research by Bovet at the Pasteur Institute in Paris in the 1930s. His work earned a Nobel prize, and in his speech, he discussed the discovery of antihistamines made in his search for histamine inhibitors. The first compound officially classed as an antihistamine by Bovet, alongside Ungar and Parrot, was piperozan in 1937[2]. This discovery and its resulting research earned Bovet the 1957 Nobel Prize for Physiology or Medicine. In 1942, the first antihistamine used in human trials was phenbenzamine, which is still used in topical anti-allergy creams and ointments. The success of this first human trial led to the discovery and development of many more antihistamines, including diphenhydramine, tripelennamine, chlorpheniramine, and promethazine. These newly formulated drugs were widely used in allergy treatments by the mid 1940's[2]. As antihistamine research continued, it became apparent that the antihistamine molecules were large, bulky compounds, while histamines themselves are relatively simple compounds. Histamine contains an imidazole ring with an ethylamine side chain. The nitrogen-containing structure of histamine led to the common inclusion of basic amino groups in antihistamines. This inclusion is still seen today, as virtually all antihistamines contain at least one nitrogen-based group.

Antihistamines in Raman Spectroscopy

In Raman spectroscopy, each molecular group has an expected mode based on the frequency of the Raman. Amine groups are expected to appear around 3150 cm^{-1} to 3480 cm^{-1}. Other groups found in many antihistamine compounds includes, but is not limited to, alcohols, alkanes, carboxyls, esters, ethers, halides, pyridyl rings, and phenyl rings. These varying functional groups have expected Raman frequencies. Alcohols exhibit weak peaks between 3210 cm^{-1} and 3250 cm^{-1}. Alkane chains appear as a moderate peak around 375 cm^{-1}. Carboxyl groups appear as a moderate peak between 1610 cm^{-1} and 1740 cm^{-1}. Esters exhibit moderate peaks in the 1710 cm^{-1} to 1745 cm^{-1} region. Ethers give weak peaks at a low frequency, ranging between 800 cm^{-1} and 950 cm^{-1}. Chlorine based halides, the most common halides in antihistamines, exhibit strong peaks between 550 cm^{-1} and 800 cm^{-1}. Aromatic rings show multiple peaks: strong at approximately 1000 cm^{-1}, moderate at approximately 1475 cm^{-1}, and strong at approximately 1580 cm^{-1}. Non-aromatic rings typically exhibit moderate to strong ,peaks between 600 cm^{-1} and 900 cm^{-1}, with the exact frequency dependent on the molecules making up the ring (Table 5).

Spectral Expectations of Pharmaceutical Excipients

The secondary compounds seen in this study are: magnesium stearate, corn starch, hypromellose, lactose monohydrate, polydextrose, polyethylene glycol, povidone, titanium dioxide, triacetin, butylparaben, gelatin, lactose anhydrous, methylparaben, polysorbate 80, silicon dioxide, microcrystalline cellulose, pregelatinized starch, and croscarmellose sodium

(Table 2). Some of these ingredients are Raman active on their own, while others act as Raman activity inhibitors when combined with other common pharmaceutical compounds.

Magnesium stearate, a compound present in each over-the-counter tablet or capsule used, is a lubricating agent. It assists in ensuring mass-produced pharmaceutical tablets each contain the same, and correct, amount of active ingredient, and assist in the separation of tablet components in the body[15]. When combined with strictly inorganic compounds, magnesium stearate has two distinct Raman bands at 1060 cm^{-1} and 2848 cm^{-1} when combined with strictly inorganic species[14]. The strong peak at 1060 cm^{-1} is not seen when the compound is in the presence of other organic species[14]. Overall, magnesium stearate appears to enhance the Raman activity of tablets. Its lubricating function prevents the individual tablet components from sticking to each other and allow a more intense scattering of photons during Raman analysis.

Corn starch is commonly used a binding agent in pharmaceutical tablets. It is known to have 3 distinct Raman active regions from previous studies: a strong peak at 2900 cm^{-1}, a set of moderate activity between 900 cm^{-1} and 1500 cm^{-1}, and a strong peak at approximately 480 cm^{-1}[16]. The strong band at 480 is the most distinct Raman activity in corn starch compared to other polysaccharides, and is typically seen when in mixture with other compounds, but can be easily "lost" when in combination with compounds that exhibit relatively stronger Raman activity within the same region[17].

Hypromellose serves as both a release-rate control agent and as an outer coating in pharmaceutical tablets[18]. It tends to inhibit Raman activity when mixed with other compounds, and has very little Raman activity on its own. Hypromellose has a very weak Raman peak at

1500 cm^{-1} and a weak, broad peak at 3000 cm^{-1} [13]. These peaks do not have enough relative intensity to visibly appear in spectra of most mixtures; they are readily overpowered by compounds with stronger Raman activity. They also decrease the activity of the other components when in a mixture, as is seen in some of the runs of this study (Figures 2,3), and as was seen in previous studies[13].

Lactose monohydrate serves as a compression aid in pharmaceutical tablets[19]. It assists in the process of forming the loose powered compounds into a solid tablet during production. Lactose monohydrate exhibits strong Raman activity between 400 cm^{-1} and 500 cm^{-1}, weak activity between 500 cm^{-1} and 800 cm^{-1}, moderate to strong activity between 800 cm^{-1} and 1200 cm^{-1}, and moderate activity between 1200 cm^{-1} and 1600 cm^{-1}. The most distinct peaks in the spectrum are seen at approximately 490 cm^{-1}, 1100 cm^{-1}, and 1250 cm^{-1}[12]. No significant effects of the presence of lactose monohydrate in a mixture have been noted in any previous studies[20], nor were any peaks seen in the experimental trials of this study consistent with those of pure lactose monohydrate.

Polydextrose is commonly used as a coating for pharmaceutical tablets. It is a sugar that is soluble in water, but unable to be digested within the body, making it ideal for covering the main body of tablet to prevent foul tastes during intake[21]. The spectral expectations of a single dextrose unit can be utilized to derive approximate expectations of a polydextrose molecule in Raman spectroscopy. Dextrose exhibits strong Raman activity between 400 cm^{-1} and 600 cm^{-1}, with a distinct peak at about 550 cm^{-1}. Moderate to weak activity is seen between 1000 cm^{-1} and 1500 cm^{-1}, with three distinct, relatively isolated peaks occurring between the two active

regions[17]. Dextrose, along with polydextrose, does not contain any doubled-bonded species within its chemical structure. This means that there is no significant Raman activity after 1500 cm^{-1}.

Polyethylene glycol serves as a lubricating agent in the formation of pharmaceutical tablets. It binds with the other components and prevents them from sticking to the machinery in production[22]. Polyethylene glycol has Raman activity that expresses as multiple distinct peaks, rather than regions of activity. Weak Raman peaks are exhibited at approximately 550 cm^{-1}, 600 cm^{-1}, 1100 cm^{-1}, and 1250 cm^{-1}. Moderate peaks are exhibited at approximately 300 cm^{-1}, 400 cm^{-1}, 1150 cm^{-1}, 1300 cm^{-1}, and 1500 cm^{-1}. At approximately 900 cm^{-1}, a strong peak is seen[17]. Reviews of a previous studies indicates that polyethylene glycol may serve as a Raman inhibitor, lowering peak intensity[23].

Povidone is utilized in pharmaceutical tablets as both a binding agent and a disintegrant, as it is both compressible and readily soluble in water[24]. Povidone exhibits two distinct regions of Raman activity in its spectrum. A strong peak is exhibited at approximately 2900 cm^{-1}. Moderate activity is exhibited between 400 cm^{-1} and 1700 cm^{-1}. The most distinct peaks in this activity region include 750 cm^{-1}, 950 cm^{-1}, 1250 cm^{-1}, 1450 cm^{-1}, and 1700 cm^{-1} [25].

Titanium dioxide is most commonly used as a UV protector in sunscreens, but is also utilized in pharmaceuticals as pigment[26]. Titanium dioxide exists in multiple forms, which include three different crystal structures and powders. The Raman spectrum of the powder form is most relevant to this study. Powdered titanium dioxide exhibits a series of three distinct peaks, at approximately 400 cm^{-1}, 515 cm^{-1}, and 640 cm^{-1}. These peaks are expected to appear in

mixtures as well[27], but no clear research was located that examined if the presence of titanium dioxide causes Raman shift in the other mixture components.

Triacetin, or glyceryl triacetate, is utilized as a solvent in the process of pharmaceutical tablet production. It contains the necessary chemical properties to completely dissolve other tablet components, and ensures an even distribution of each component throughout the tablet[28]. Triacetin has 4 distinct Raman bands at 530 cm^{-1}, 900 cm^{-1}, 1750 cm^{-1}, and 2950 cm^{-1}. Between 200 cm^{-1} and 1500 cm^{-1}, weak to moderate activity is generally exhibited[29]. In mixtures, the only peaks expected to appear are at approximately 900 cm^{-1} and 1750 cm^{-1}, based on previous studies[30].

Butylparaben serves as a suspending agent in medications[31]. It helps to ensure that the active ingredients stay evenly distributed throughout the mixture when tablets are being manufactured. Pure butylparaben primarily exhibits Raman activity between 500 cm^{-1} and 1700 cm^{-1}. Distinct peaks in its spectrum are exhibited at 650 cm^{-1}, 900 cm^{-1}, 1250 cm^{-1}, 1600 cm^{-1}, and 1700 cm^{-1} [(32)]. Reviewing previous research, it appears that butylparaben does not affect the overall spectrum when in a mixture. The butylparaben bands grow weaker in intensity with a lower relative concentration in the mixture[33].

Gelatin is utilized in pharmaceuticals to form capsule shells and coatings on tablets[34]. Pure gelatin exhibits weak Raman activity between 800 cm^{-1} and 1700 cm^{-1}, and a distinct, moderate, broad peak at approximately 2950 cm^{-1}. These peaks have very little, if any, effect on the spectrum of a mixture containing gelatin. In low relative concentrations, the Raman bands of gelatin will likely not be seen[35].

Lactose anhydrous is utilized in pharmaceuticals as a binding agent in tablets, as it is very compressible[36]. Its Raman activity is almost synonymous with the activity of the other forms of crystalline lactose, such as lactose monohydrate. Therefore, both forms exhibit virtually the same Raman bands, with moderate to weak activity between 400 cm^{-1} and 800 cm^{-1}, moderate to strong activity between 800 cm^{-1} and 1200 cm^{-1}, and moderate activity between 1200 cm^{-1} and 1600 cm^{-1}. The most distinct peaks in the spectrum are seen at approximately 490 cm^{-1}, 1100 cm^{-1}, and 1250 cm^{-1}[1(12)].

Methylparaben serves as a preservative in pharmaceutical industry, as well as in food and cosmetics[37]. Pure methylparaben exhibits Raman activity in two distinct regions, 400 cm^{-1} to 1700 cm^{-1} and 2900 cm^{-1} to 3100 cm^{-1}. The lower wavelength region has strong peaks at approximately 650 cm^{-1}, 850 cm^{-1}, 1150 cm^{-1}, 1300 cm^{-1}, 1600 cm^{-1}, and 1700 cm^{-1}. Two distinct peaks at approximately 2975 cm^{-1} and 3100 cm^{-1} are seen in the upper region of the spectrum[38]. In mixtures, methylparaben behaves similarly to butylparaben, and its Raman activity loses intensity as the compound's relative concentration decreases[33].

Polysorbate 80, commonly called by one of its manufactures, Tween 80, is a solubility agent and excipient in pharmaceutical tablets[39]. As a general class of compounds, polysorbates exhibit weak Raman activity. All polysorbates exhibit sparse Raman activity between 800 cm^{-1} and 1500 cm^{-1}. Polysorbate 80 contains an identifying and unique peak at approximately 1650 cm^{-1}. This peak is often utilized to identify the presence of polysorbate 80, especially in comparison to other grades of polysorbate[40].

Silicon dioxide, also commonly known as colloidal silicon dioxide and silica, is utilized for multiple features in pharmaceutical tablets. It is most commonly used as an anti-caking agent, adsorbent, disintegrant, or glidant. These properties primarily affect the actual manufacturing of the tablet, rather than how the tablet functions and responds within the body[41]. Silicon dioxide exhibits three Raman bands, one of which is sharp and distinct and the other two are broad. The sharp peak is exhibited at approximately 520 cm^{-1}, a moderately broad peak at 1250 cm^{-1}, and a flat, broad peak centered at approximately 2800 cm^{-1}. The overall width of the high-wavelength, broad peak is often attributed to general electronic Raman scattering in silicon samples[42].

Microcrystalline cellulose, a refined wood pulp, is utilized as a compressible dissolving agent in pharmaceutical tablets. Despite not being degraded during digestion, compact mixtures containing microcrystalline cellulose break apart quickly in the body[43]. Microcrystalline cellulose exhibits Raman activity in three regions, 300 cm^{-1} to 600 cm^{-1}, 1000 cm^{-1} to 1500 cm^{-1}, and 2800 cm^{-1} to 3000 cm^{-1}. Distinct peaks are noted at 1100 cm^{-1}, 1380 cm^{-1}, 1500 cm^{-1}, and 3000 cm^{-1}. In comparison to other forms of cellulose, such as hypromellose and methyl cellulose, the Raman spectra alone is virtually indistinguishable. Differences in Raman bands are very small, and require in-depth analysis[44].

Pregelatinized starch is utilized in the pharmaceutical industry as a diluent, disintegrant, glidant, and binder. Starches absorb was rapidly, allowing for quick disintegration of tablets. They also assist in preventing tablets from sticking to machinery during manufacturing[45]. Pregelatinized starch is the product formed when regular starches are broken down and

irreversibly dissolved into water. Partially pregelatinized starch, which is most common in the pharmaceutical industry, is then heated to dryness, returning the starch to a solid, granule form[46]. Waxy, wild-type, and pregelatinized starches all exhibit similar Raman spectroscopic properties. A strong peak is exhibited at approximately 500 cm^{-1}, and moderate activity is exhibited between 800 cm^{-1} and 1500 cm^{-1}. A strong, broad peak is also seen at approximately 2925 cm^{-1}. This patterns of activity are virtually identical in all forms of starch[46].

Croscarmellose sodium is a disintegrant in pharmaceutical tablets. It assists in facilitating the breakdown of a sealed tablet once it reaches the intestinal tract[48]. On its own, croscarmellose sodium exhibits very weak Raman activity. It exhibits a small series of peaks between 1000 cm^{-1} and 1500 cm^{-1}, and a single peak at approximately 2900 cm^{-1}. Weak, broad peaks are seen at approximately 1100 cm^{-1}, 1300 cm^{-1}, and 1400 cm^{-1} in the low wavenumber region. When in a mixture of other compounds, croscarmellose sodium has little no effect on the Raman spectrum. This is especially true when analyzing pharmaceutical tablet spectra[49].

Methods and Materials

Antihistamines Utilized in this Study

Multiple over-the-counter antihistamines were utilized in this research. They were all purchased as over the counter anti-allergy tablets or capsules. These specific antihistamines included loratadine (Walgreens, Claritin), diphenhydramine hydrochloride (Walgreens),

chlorpheniramine maleate (Walgreens), cetirizine hydrochloride (Walgreens, Duane Reade, Assured), and fexofenadine hydrochloride (Walgreens).

Experimental Procedure

Experimental Raman spectra were first generated on a MiniRam Portable NIR Raman Spectrometer (B&W Tek, Inc.). Data was collected through the BWSpec software on a paired computer. This Raman spectrometer utilized a 16-bit digitizer, thermoelectrically cooled 2048 pixel charge-coupled device,, and a 785 nm narrow linewidth laser[6].

Experimental Raman spectra were also generated on a JASCO NRS-3100 confocal dispersive Raman spectrometer equipped with a micro Raman assembly (Easton, MD). Raman scattering was induced with a 12 mW 488 nm laser and collected with a thermoelectrically cooled charge-coupled device detector. Solid samples were prepared on quartz slides and liquid samples in a quartz cuvette. The trials were run at room temperature.

Theoretical Procedure

Theoretical spectra were calculated with Gaussian 09 software. The chemical structures of each compound were constructed in GaussView[3]. The geometry of the molecule was optimized, and vibrational frequencies were performed with the density functional theory approximation, utilizing the Becke's three-parameter exchange function in combination with Lee, Yang, and Parr correlation function[4,5]. Each calculation was run under the 6-31G basis set and analyzed in Gaussview05.

Results and Discussion

Cetirizine Theoretical Spectrum

Theoretically, cetirizine exhibits weak to moderate Raman activity between 0 cm^{-1} and 1800 cm^{-1}, and moderate to strong activity between 2800 cm^{-1} and 4000 cm^{-1} (Figure 1). The most significant peaks in the lower activity region were 830 cm^{-1}, 1040 cm^{-1}, 1550 cm^{-1}, and 1660 cm^{-1}. The peak and surrounding weak activity at 830 cm^{-1} can be attributed to the ether functional group. The activity between 1000 cm^{-1} and 1575 cm^{-1} is caused by the multiple aromatic rings present within cetirizine's structure. At 1660 cm^{-1}, carboxylic acid causes the moderately strong peak. In the high-wavenumber range, the most prominent Raman peaks are seen at 2960 cm^{-1}, 3010 cm^{-1}, 3110 cm^{-1}, 3240 cm^{-1}, and 3600 cm^{-1}. The two strong peaks at 2960 cm^{-1} and 3110 cm^{-1} are attributed to the two aromatic rings within cetirizine. The moderate peak at 3010 cm^{-1} and the moderate to strong peak at 3600 cm^{-1} can both be attributed to the alcohol within the carboxylic acid. The strong peak at 3240 cm^{-1} is indicative of the piperazine-like ring within the molecular structure.

Chlorpheniramine Theoretical Spectrum

The theoretical, optimized Raman spectrum of chlorpheniramine exhibited strong Raman activity in the 2900 cm^{-1} to 3300 cm^{-1} region (Figure 1). Weak to moderate activity was exhibited up to 1575 cm^{-1}. A moderate peak was also seen at 3600 cm^{-1}. Chloropheniramine's most prominent functional groups include chlorophenyl, dimethyl amine, and pyridine. The peak at 3600 cm^{-1} is most characteristic of the dimethyl amine side chain, as amines are broadly

estimated to fall between 3100 and 3600 cm^{-1}. The moderate to strong activity between 2900 cm^{-1} and 3250 cm^{-1} can be attributed to the presence of the pyridine ring, with the double-bonded ring nature shifting the usual nitrogen-carbon bond activity area to a slightly higher frequency. This area of strong Raman activity is also influenced by the chlorophenyl, as benzene rings are estimated to appear around 2900 cm^{-1} to 3100 cm^{-1}. The weak activity from 100 cm^{-1} to 1200 cm^{-1} is caused by the influence and nature of the carbon chains within the compound. The relatively stronger peak at 1660 cm^{-1} is a result of the nitrogen atom within the pyridine ring, as that falls into the characteristic frequency range of carbon-nitrogen double-bonded atoms.

Diphenhydramine Theoretical Spectrum

Diphenhydramine contains many varying functional groups, which include diphenyl, dimethyl amine, and an ether. The theoretical spectrum of diphenhydramine is presented in figure 1. It exhibited weak Raman activity between 60 cm^{-1} and 1000 cm^{-1}, a moderate peak at 1040 cm^{-1}, weak activity between 1200 cm^{-1} and 1600 cm^{-1}, and a moderate peak at 1660 cm^{-1}. The spectra also showed moderate activity between 2900 cm^{-1} and 3200 cm^{-1}, a strong peak at 3226 cm^{-1}, and two moderate peaks at 3532 cm^{-1} and 3649 cm^{-1}. The weak activity from 60 cm^{-1} to 600 cm^{-1} is primarily attributed to the carbon chains within the molecule. The weak to moderate activity between 650 cm^{-1} and 1000 cm^{-1} stems from the ether group within the compound. The distinct peak at 1040 cm^{-1} is a result of the two benzene rings, or the diphenyl group. The peak at 1660 cm^{-1} is also attributed to the phenyl rings. The strong Raman activity between 2900 cm^{-1} and 3200 cm^{-1} and the distinct peaks at 3226 cm^{-1}, 3532 cm-1, and 3649 cm-1, are indicative of the dimethyl amine.

Fexofenadine Theoretical Spectrum

Pure fexofenadine theoretically exhibits weak to moderate Raman activity between 0 cm^{-1} and 1700 cm^{-1}. Moderate to strong Raman activity is seen between 3000 cm^{-1} and 3700 cm^{-1} (Figure 1). The most distinct peaks in the lower wavenumber region include 50 cm^{-1}, 391 cm^{-1}, 1039 cm^{-1}, 1252 cm^{-1}, 1364 cm^{-1}, 1408 cm^{-1}, 1484 cm^{-1}, 1543 cm^{-1}, and 1669 cm^{-1}. Peaks at very low wavenumbers, typically under 400 cm^{-1}, are considered part of the fingerprint region, which is a result of all carbon-carbon bonds within the molecule. The peak at 1484 cm^{-1} can be attributed to methylene groups within the molecule. The moderate peaks at 1039 cm^{-1}, 1252 cm^{-1}, 1364 cm^{-1}, and 1408 cm^{-1} are indicative of the aromatic rings within the molecule. The similarly-located peak at 1543 cm^{-1} is indicative of molecular rings in general. The presence of the carboxylic acid within fexofenadine is illustrated by the moderate peak at 1669 cm^{-1}. In the upper wavenumber region, the most distinct peaks include 3019 cm^{-1}, 3064 cm^{-1}, 3226 cm^{-1}, and 3604 cm^{-1}. The two peaks at 3019 cm^{-1} and 3064 cm^{-1} represent the aromatic rings, likely the diphenyl group with their close wavenumbers. The two upper peaks, 3226 cm^{-1} and 3604 cm^{-1}, are indicative of the nitrogen-containing groups within the molecule.

Loratadine Theoretical Spectrum

Pure loratadine theoretically exhibits weak to moderate Raman activity between 300 cm^{-1} and 1600 cm^{-1}, and moderate to strong Raman activity between 2800 cm^{-1} and 3300 cm^{-1} (Figure 1). In the lower region of activity, the most distinct peaks include 556 cm^{-1}, 652 cm^{-1}, 740 cm^{-1}, 1028 cm^{-1}, 1108 cm^{-1}, 1308 cm^{-1}, 1524 cm^{-1}, and 1628 cm^{-1}. The peaks at 556 cm^{-1}, 652 cm^{-1}, and 740 cm^{-1} coincide with the stretching of the chlorine bond. The peaks at 1028 cm^{-1}, 1109 cm^{-1},

1308 cm^{-1}, and 1524 cm^{-1} all correspond to the multiple rings within the structure. Loratadine contains four cyclic rings, which is consistent with the four peaks within the expected Raman activity wavenumber range. The moderate peak at 1628 cm^{-1} indicates the ester within the molecule. Looking at the upper range of Raman activity, distinct peaks are exhibited at 2956 cm^{-1}, 3060 cm^{-1}, 3140 cm^{-1}, 3228 cm^{-1}, and 3268 cm^{-1}. The peaks at 2956 cm^{-1} and 3060 cm^{-1} correspond to the two aromatic groups, phenyl and pyridine. The moderately strong peak at 3140 cm^{-1} indicates the amine present between the ester and carbon rings. The two peaks at 3228 cm^{-1} and 3268 cm^{-1} correlate the the carbon-hydrogen stretching of the phenyl attached the the chlorine atom. The presence of the chlorine gives the two surrounding carbons distinct vibrational motions.

Cetirizine Experimental Spectrum

Walgreens brand cetirizine tablet were utilized to generate the experimental spectrum. Cetirizine had moderate to strong Raman activity between 350 cm^{-1} and 650 cm^{-1} (Figure 2). The most distinct peak in this range include 400 cm^{-1}, 518 cm^{-1}, and 641 cm^{-1}. Weak Raman activity was exhibited between 1100 cm^{-1} and 1500 cm^{-1} (Figure 3). This region included peaks at 1145 cm^{-1}, 1237 cm^{-1}, 1284 cm^{-1}, 1447 cm^{-1}, and 1485 cm^{-1}. The only peak that is similar to the theoretical cetirizine spectrum is at 1237 cm^{-1}, attributed to the aromatic rings within the molecule. All other peaks will stem from the other tablet components, such as corn starch, which has Raman activity at 480 cm^{-1} and 2910 cm^{-1} in previous studies[11]. Lactose monohydrate, another tablet component, has moderate Raman activity between 800 cm^{-1} and 1200 cm^{-1}, making it a likely candidate for the source of the experimental activity seen[12]. The three distinct

peaks at 400 cm^{-1}, 518 cm^{-1}, and 641 cm^{-1} can likely be attributed to titanium dioxide. They appear at the same wavenumbers as expected of titanium dioxide from previous studies[27], and are mirrored in the experimental spectrum of the fexofenadine tablets, which also contain the compound.

Chlorpheniramine Experimental Spectrum

The experimental spectrum of chlorpheniramine was generated with Walgreens brand tablets. Each tablet contained four milligrams of the antihistamine, and was otherwise made up of binders and release agents. Chlorpheniramine did not show any significant Raman activity (Figures 2 and 3). This lack of activity is likely caused by the secondary, inactive ingredients in the tablet. One of the secondary compounds could serve as an Raman activity inhibitor. Chlorpheniramine tablets also contained the lowest concentration of antihistamine compared to the others studied - only four milligrams per tablet versus the next lowest of ten milligrams per tablet. This low concentration could have caused Raman activity that was too weak to be detected by the instrument.

Diphenhydramine Experimental Spectrum

This spectrum was generated with Walgreens' diphenhydramine capsules. Multiple capsules were broken open and the contents combined into a small glass vial, allowing the Raman laser to reach the sample. The sample exhibited weak to moderate Raman activity between 400 cm^{-1} and 1000 cm^{-1}. Weak Raman activity was also seen around 1600 cm^{-1}. The distinct peak for diphenhydramine include 442 cm^{-1}, 481 cm^{-1}, 622 cm^{-1}, 654 cm^{-1}, 841 cm^{-1}, 1006 cm^{-1}, and 1603 cm^{-1}. Theoretically, diphenhydramine has an ether peak at 924 cm^{-1}. One

or both of the experimental peaks at 841 cm^{-1} and 1006 cm^{-1} are likely also a result of the ether group. Diphenhydramine capsules also contain corn starch, butylparaben, gelatin, lactose anhydrous, magnesium stearate, methylparaben, polysorbate 80, and silicon dioxide (Table 2). Lactose anhydrous, similarly to lactose monohydrate, exhibits strong Raman activity between 400 cm^{-1} and 500 cm^{-1}, moderate activity between 800 cm^{-1} and 1400 cm^{-1}, and strong activity between 1600 cm^{-1} and 1800cm^{-1} [12]. Lactose anhydrous is one of the major secondary components, and is most likely to appear in the capsule's Raman spectrum. Corn starch has a strong Raman peak at 1700 cm^{-1}, making it the most likely cause of the capsule peak at 1603 cm^{-1}. Starch also exhibits a moderate peak around 450 cm^{-1}, which could be a possible source of the low wavenumber activity in the capsule's spectrum[11].

Fexofenadine Experimental Spectrum

Walgreens' fexofenadine tablets exhibit moderate Raman activity between 400 cm^{-1} and 650 cm^{-1}. Weak to moderate activity was seen between 800 cm^{-1} and 1600 cm^{-1}. The distinct peaks included 400 cm^{-1}, 519 cm^{-1}, 642 cm^{-1}, 1122 cm^{-1}, 1155 cm^{-1}, 1270 cm^{-1}, 1371 cm^{-1}, 1457 cm^{-1}, and 1675 cm^{-1} (Figure 2,3). The fexofenadine compound itself contains multiple aromatic rings and methylene chains, along with alcohols, carboxylic acids, and piperidine groups. Only the aromatic rings and the methylene chains appear in the experimental spectrum. The experimental peaks at 1270 cm^{-1} and 1371 cm^{-1} correspond to the theoretical peaks at 1252 cm^{-1} and 1364 cm^{-1}. Both of these theoretical Raman spectrum peaks correlate to the presence of aromatic rings. The tablet contained multiple secondary ingredients: colloidal silicon dioxide, croscarmellose sodium, hypromellose, lactose monohydrate, magnesium stearate,

microcrystalline cellulose, polyethylene glycol, povidone, and titanium dioxide. The Raman spectrum of the tablet displayed three distinct peaks at 924 cm^{-1}, 1020 cm^{-1}, and 1100 cm^{-1}. These peaks are mirrored in the cetirizine tablet spectrum. The peak series correlates with the expectations of titanium dioxide. The mirroring of the peaks and shared presence with cetirizine confirms that these peaks can be assigned to titanium dioxide. Fexofenadine tablets also contain lactose monohydrate, which is known to have moderate Raman activity between 800 cm^{-1} and 1200 cm^{-1} [12], possibly serving as the source of the tablet's Raman peaks at 1122 cm^{-1} and 1155 cm^{-1}. The presence of the hypromellose lessened the Raman intensity of the tablet as a whole, as it has been determined to be an inhibitors in previous studies[13].

Loratadine Experimental Spectrum

Loratadine tablets showed very little Raman activity. The only peak seen was around 1090 cm^{-1}. Similarly to chlorpheniramine, it is assumed that this lack of activity is caused by the inactive components of the tablet, the binders and releasing agents. Loratadine also had a relatively low concentration, however, the cetirizine tablet had the same concentration and showed considerably more Raman activity, making it unlikely that the low loratadine activity was due to concentration.

Comparison of Cetirizine Tablets

Cetirizine tablets of three manufacturers were analyzed via Raman spectroscopy and compared. The Walgreen's tablet, which were also used in the other parts of this study, contained secondary ingredients of corn starch, hypromellose, lactose monohydrate, magnesium stearate, polydextrose, polyethylene glycol, povidone, titanium dioxide, and triacetin. The

Duane Reade tablets, manufactured by PACK Pharmaceuticals, contained inactive ingredients of hypromellose, lactose, magnesium stearate, maize starch, polyethylene glycol, povidone, and titanium dioxide. The Assured tablets, sold exclusively by Dollar Tree[9], contained colloidal silicon dioxide, hypromellose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyethylene glycol, povidone, sodium starch glycolate, and titanium dioxide (table 3).

The Assured and Walgreen tablets exhibited similar Raman spectra, although the Assured tablets showed very little Raman activity. The Duane Reade tablets had a Raman baseline that followed in the opposite direction of the other two brands, forming a spectrum that resembles a decreasing linear function (Figure 4). This sample is the only brand and antihistamine type that exhibited this type of spectrum, and multiple tablets were analyzed to ensure accuracy. All three brands of cetirizine tablets shared the inactive ingredients hypromellose, lactose, magnesium stearate, polyethylene glycol, povidone, and titanium dioxide. It does not appear that these components had a significant effect on the tablet's spectrum. The Duane Reade and Walgreen tablets also share the presence cornstarch; the Assured and Walgreen tablets do not share any other components. The extreme difference in appearance of the Duane Reade and Walgreen tablets suggests that the cornstarch does not have an effect on the Raman activity.

The Walgreen tablet included polydextrose and triacetin, the only tablet analyzed to have these components. As the Walgreen tablet was the only tablet to contain significant Raman activity, the data strongly suggests that the peaks visualized correspond to the Raman activity of polydextrose or triacetin. The Assured tablets uniquely contained colloidal silicon dioxide, microcrystalline cellulose, and sodium starch glycolate. The spectrum of the Assured cetirizine

tablet did not contain significant Raman bands, indicating that one of these unique components act as an inhibitor to Raman activity. Pure cetirizine theoretically exhibits a Raman peak at 1236 cm^{-1}, which is possibly reflected in the Walgreen tablet, which showed a weak Raman band at 1237 cm^{-1} (Figures 1, 4).

Comparison of Loratadine Tablets

Walgreen and Claritin loratadine tablets each followed the same general trend in Raman activity, but only the Claritin exhibited significant Raman bands (Figure 5). Both tablets contained lactose monohydrate and magnesium stearate, which likely contributes to the overall appearance of the spectra. The Walgreen tablet also contained povidone and pregelatinized starch. The Claritin tablet contained corn starch (Table 4). Based on previous studies, both pregelatinized and corn starches exhibit very similar, if not the same, Raman properties[46]. Neither is recorded to significantly affect the spectrum of a mixture, such as a tablet. This indicates that the povidone is the most likely component to have decreased the Raman activity of the Walgreen spectra, as it is the only unique ingredient.

Theoretical loratadine is expected to show Raman bands at 740 cm^{-1}, 1028 cm^{-1}, 1308 cm^{-1}, 1524 cm^{-1}, and 1628 cm^{-1}, in the wavenumber range utilized for the loratadine tablet comparisons (Figure 1). The peak at 1028 cm^{-1} is possibly reflected in both the Walgreen and Claritin tablets, which both exhibited a Raman band at approximately 1090 cm^{-1} (Figure 5). However, magnesium stearate also theoretically exhibits a Raman peak in this wavenumber area, at 1060 cm-1[(14)]. This compound is present in both tablets. Despite the two possibilities for the origin of the Walgreen and Claritin Raman peaks, it is more likely that the peak can be attributed to the loratadine. Magnesium stearate is present in all of the Walgreen tablets of varying active

ingredients tested (Table 2), and a Raman band surround 1060 cm-1 was not present in all of the experimental spectra (Figure 3).

Conclusions

Raman activity of active ingredients in over-the-counter antihistamine tablets is severely inhibited by the secondary ingredients. Theoretically, all of the antihistamines studied exhibit clear, high Raman activity. However, when experimentally analyzed, many of the tablets contained very little to no Raman activity. Analysis of the effects of the secondary, inactive ingredients gave insight to the causes of this Raman inhibition.

Throughout this study, it was confirmed that hypromellose and polyethylene glycol suppress the Raman activity in a mixture. It is suspected that lactose, both in monohydrate and anhydrous form, may also suppress the effects of Raman scattering, but further study is required to confirm or deny this claim. Hypromellose appears to have the greatest effect on Raman activity, as its presence in the cetirizine and fexofenadine tablets appeared to overpower the presence of other compounds (Figure 2).

Many of the excipients also held fluorescent properties, which also inhibit the appearance of Raman activity on the spectrum. The fluorescence of the secondary ingredients over-excites the Raman-laser photons, which will then carry significantly higher energy than the photons in the normal Raman activity range. These high-energy molecules prevent the spectrometer from accurately measuring and recording the sample's Raman activity.

The relative concentration of active to inactive ingredients in a tablet also appeared to have a large effect on the Raman activity. The chlorpheniramine tablet, with the lowest

concentration of antihistamine, exhibited very little to no Raman activity. Fexofenadine tablets, with almost forty times more histamine content than the chlorpheniramine tablet, exhibited the most Raman activity, primarily ranging from moderate to strong bands.

Figures and Tables

Table 1. Antihistamine compounds used in this study.

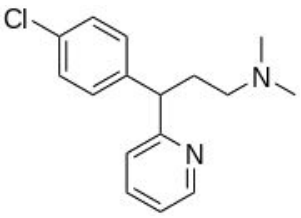
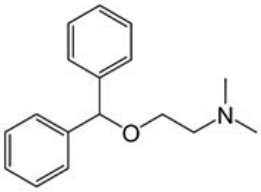
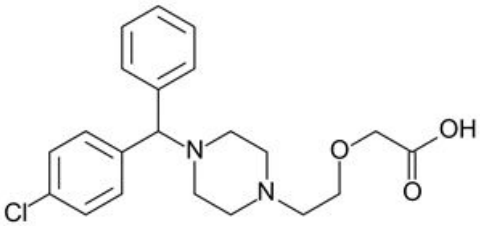
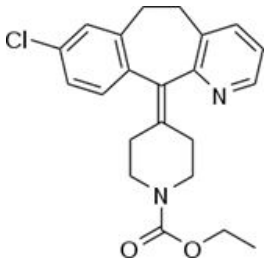
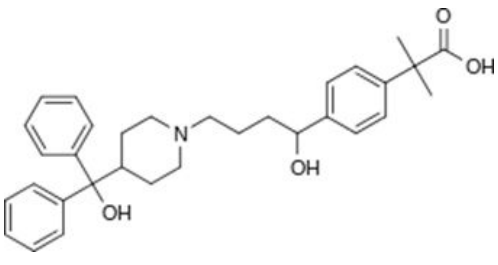
Compound	Structure
Chlorpheniramine	
Diphenhydramine	
Cetirizine	
Loratadine	
Fexofenadine	

Table 2. Tablet and capsule components of each antihistamine type, excluding dyes[7].

Antihistamine	Inactive Ingredients (Walgreens)
Cetirizine (10mg)	Corn Starch, Hypromellose, Lactose Monohydrate, Magnesium Stearate, Polydextrose, Polyethylene Glycol, Povidone, Titanium Dioxide, Triacetin
Diphenhydramine (25mg)	Corn Starch, Butylparaben, Gelatin, Lactose Anhydrous, Magnesium Stearate, Methylparaben, Polysorbate 80, Silicon Dioxide
Chlorpheniramine (4mg)	Anhydrous Lactose, Corn Starch, Magnesium Stearate, Microcrystalline Cellulose
Loratadine (10mg)	Lactose Monohydrate, Magnesium Stearate, Povidone, Pregelatinized Starch
Fexofenadine (150mg)	Colloidal Silicon Dioxide, Croscarmellose Sodium, Hypromellose, Lactose Monohydrate, Magnesium Stearate, Microcrystalline Cellulose, Polyethylene Glycol, Povidone, Titanium Dioxide

Table 3. Cetirizine tablet components of each brand used for Raman comparison[7,9,10].

Cetirizine Brand Comparison	
Brand	**Inactive Ingredients**
PACK Pharmaceuticals (via Duane Reade)	Hypromellose, Lactose, Magnesium Stearate, Maize Starch, Polyethylene Glycol, Povidone, Titanium Dioxide
Walgreens	Corn Starch, Hypromellose, Lactose Monohydrate, Magnesium Stearate, Polydextrose, Polyethylene Glycol, Povidone, Titanium Dioxide, Triacetin
Assured	Colloidal Silicon Dioxide, Hypromellose, Lactose Monohydrate, Magnesium Stearate, Microcrystalline Cellulose, Polyethylene Glycol, Povidone, Sodium Starch Glycolate, Titanium Dioxide

Table 4. Loratadine tablet components of each brand used for Raman comparison[7,8].

Loratadine Brand Comparison	
Brand	**Inactive Ingredients**
Walgreens	Lactose Monohydrate, Magnesium Stearate, Povidone, Pregelatinized Starch
Claritin	Corn Starch, Lactose Monohydrate, Magnesium Stearate

Table 5. Raman peak frequencies of functional groups commonly found in antihistamines.

RAMAN Band Correlation Table		
Approximate Wavenumber Range (cm^{-1})	***Group***	***Relative Intensity***
250-400	C-C aliphatic chain	Strong
550-790	C-Cl	Strong
800-950	C-O-C	Weak
990-1100	Aromatic Rings	Strong
1450-1505	Aromatic Rings	Moderate
1550-1610	Aromatic/hetero ring	Strong
1550-1700	Amide	Strong
1600-1710	Ketone	Moderate
1610-1740	Carboxylic Acid	Moderate
1710-1745	Ester	Moderate
2680-2740	Aldehyde	Weak
2750-2800	N-CH3	Weak
2870-3100	Aromatic C-H	Strong
2880-3530	OH	Weak
3150-3480	Amide	Moderate
3150-3480	Amine	Moderate
3200-3400	Phenol	Weak
3210-3250	Alcohol	Weak

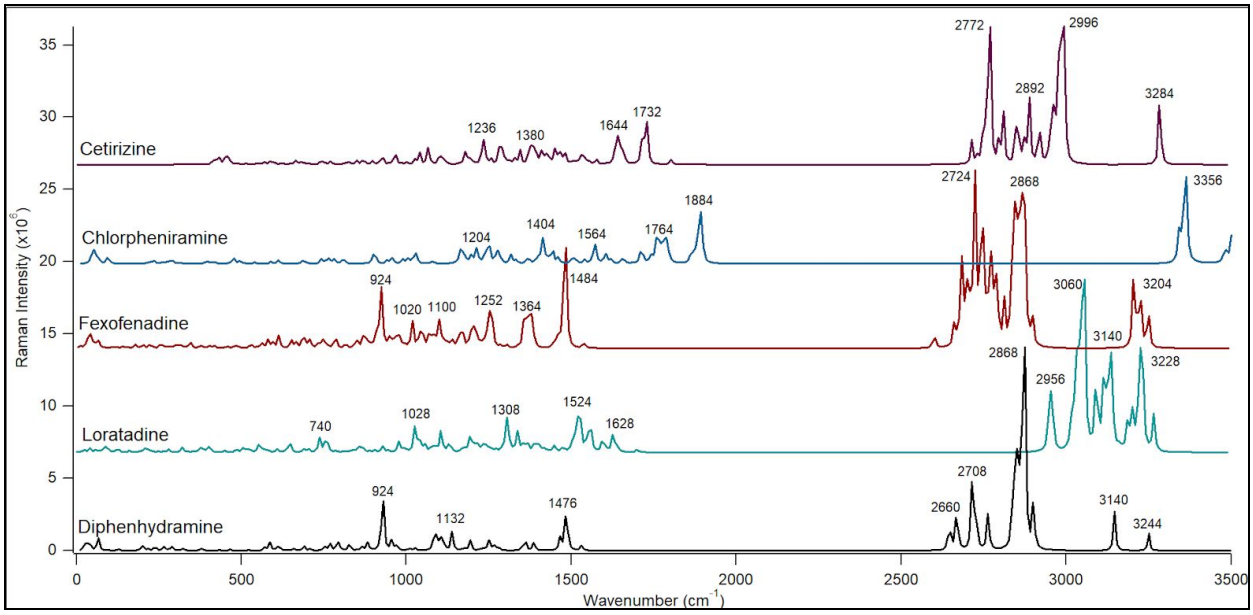


Figure 1. Theoretical Raman spectrum of each antihistamine studied (Walgreen), calculated on Gaussian 09.

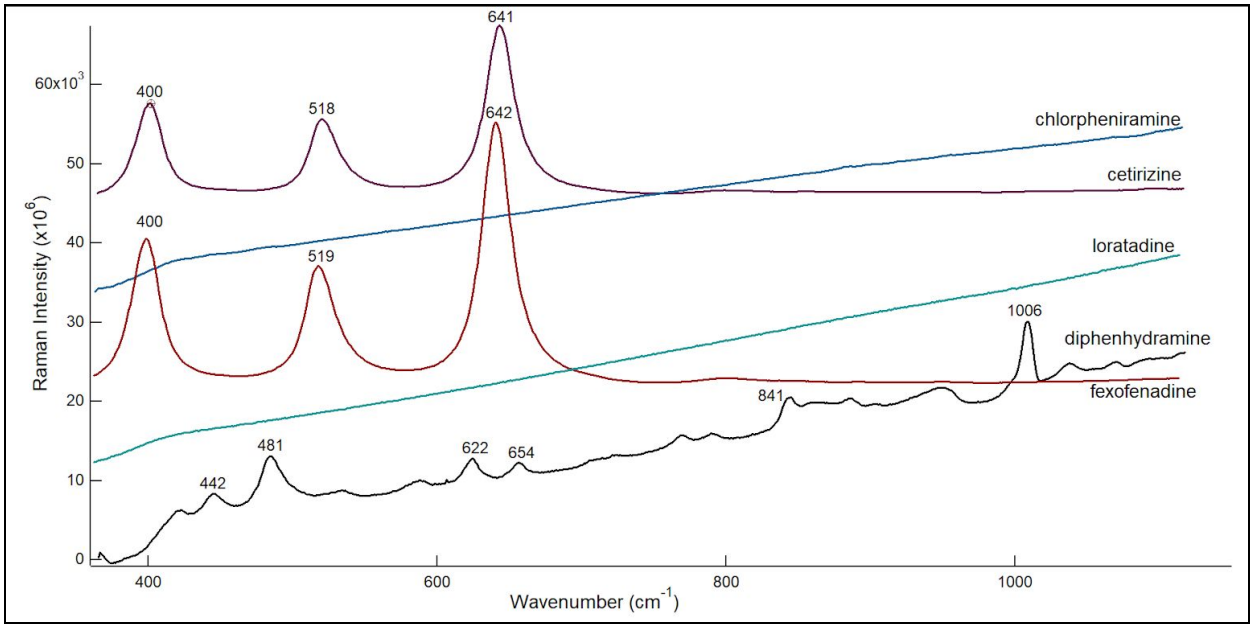


Figure 2. Experimental Raman spectra of each antihistamine (Walgreen) at the lower wavenumber region.

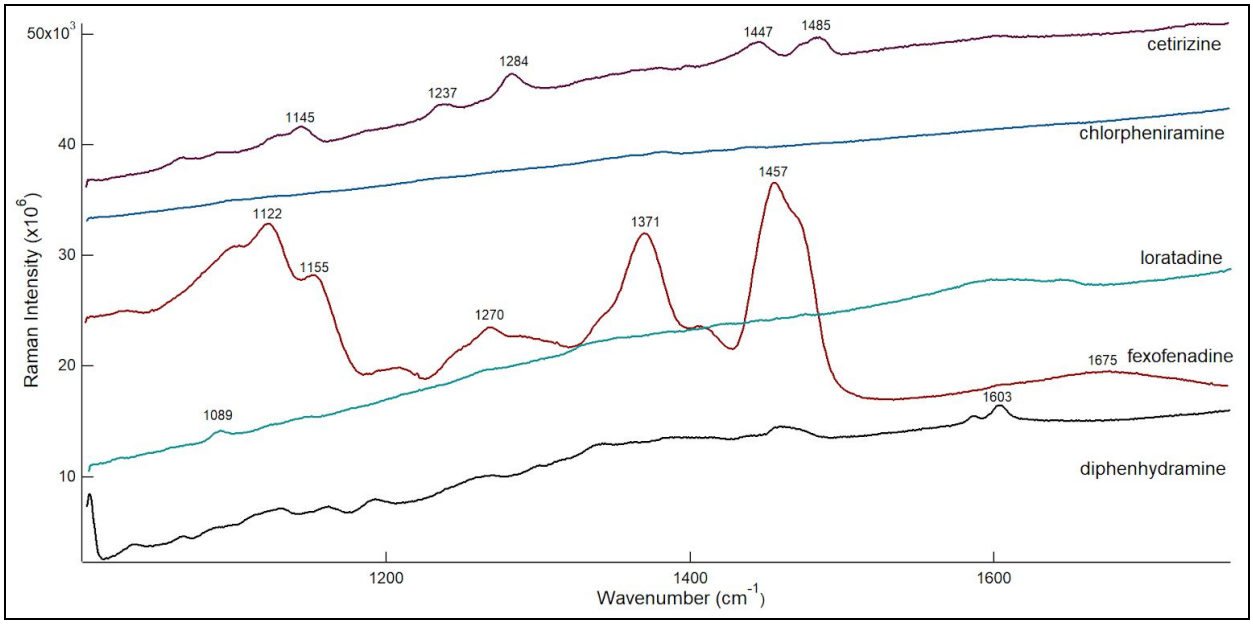


Figure 3. Experimental Raman spectra of each antihistamine (Walgreens brand) at the higher wavenumber region.

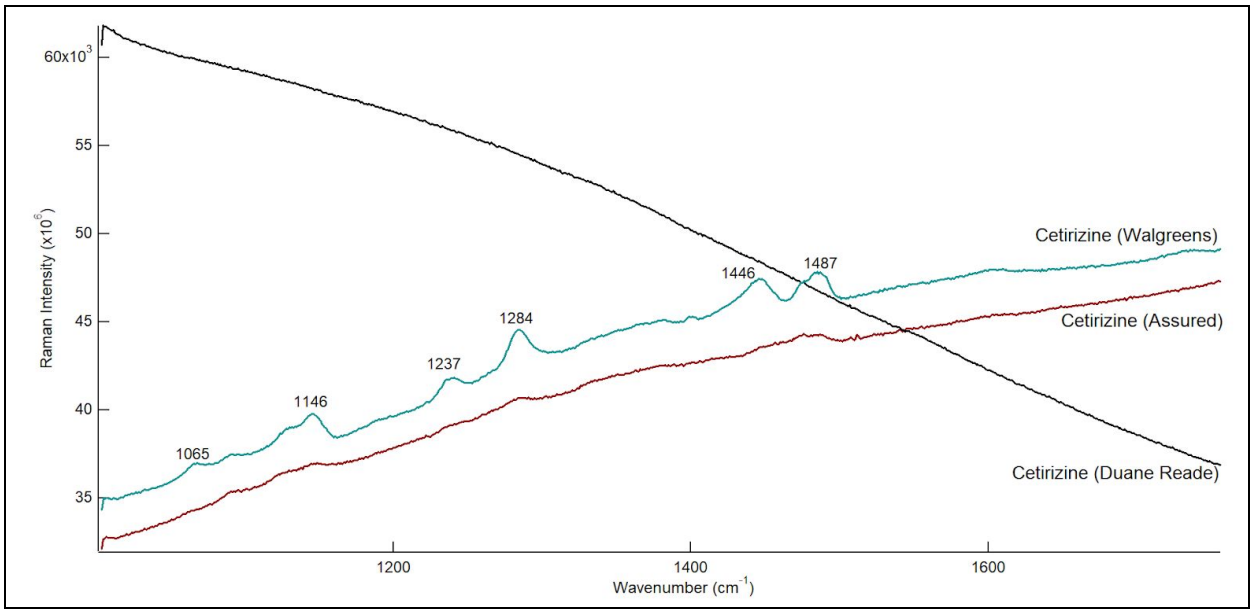


Figure 4. Experimental Raman spectra of three brands of cetirizine tablets.

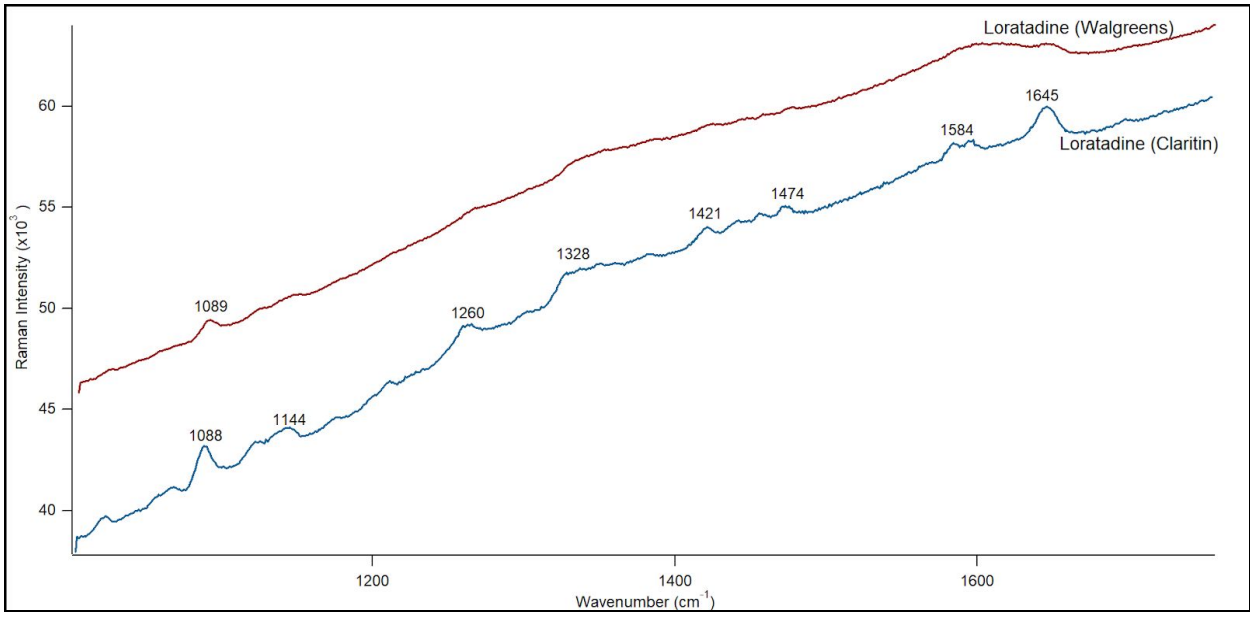


Figure 5. Experimental Raman spectra of two brands of loratadine tablets.

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