Vibrational analysis of acetaminophen from commercial tablets

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Abstract. Acetaminophen (AAP) is an active ingredient very used in many pharmaceutical preparations. AAP is a pain reliever and a fever reducer drug. Generally, it is used to treat many conditions such as headache, muscle aches, arthritis, backache, toothaches, colds, and fevers. Standard methods most used for AAP chemical detection are based on chromatography and spectrophotometry techniques, these methods generally imply a sample preparation step, while vibrational spectroscopy based methods do not. Hence, analytical methods based on vibrational spectroscopy are very important for the pharmaceutical industry and law enforcement agency, given that allow obtain a way easy and fast molecular information for its detection. An effort for attaining a reliable identification procedure for the qualitative determination of AAP in different pharmaceuticals product, an additional statistical treatment of ATR-FTIR data is proposed. The proposed method was tested on solid samples containing API. The statistical routine of Hit Quality Index (HQI) values yielded excellent results. The results show that ATR-FTIR, QCL and Raman spectra are useful for detection of AAP in the drugs tested. Using the proposed method allowed to know if the drug is present as a desired product or it is counterfeit drugs.

1. Introduction
Acetaminophen (AAP) or paracetamol is one of the most used active ingredient in pharmaceutical formulations. AAP is generally used as an analgesic and antipyretic to relieve pain and reduce fever, it is used specifically to treat various conditions such as headache, muscle aches, arthritis, back pain, toothaches, colds and fevers. The amount provided in the patient is of the utmost importance, insufficient could prolong the treatment of a certain condition, and even worse, a lot could have adverse consequences for the patient's health.

Due to its easy availability without prescription, your deliberate consumption or overdose can happen. Overdoses of AAP are associated with liver toxicity and renal failure. Liver toxicity begins with plasma levels of AAP in the range of 120 μgmL⁻¹ 4 h after ingestion and severe damage occurs with plasma levels of up to 200 μgmL⁻¹ after 4 h ingestion [1]. Many analytical methods have been used and proposed by the scientific community for the determination of AAP, both for pharmaceutical formulations and for biological samples. Among them, chromatographic [2] and optical (UV-VIS) [3] methods are the most used. Other analytical methods such as electrochemicals [4] have recently been reported by the scientific community. These techniques have been shown to be efficient in terms of...
reproducibility and obtaining low detection limits. However, in their chemical analysis process, sample
preparation steps are carried out by lengthening the analysis time and making it difficult to use in the
field.

In recent years, analytical methods based on the use of special infrared (IR) and Raman spectroscopy
have been shown to be useful for the analysis of different chemical compounds, including drugs in
different pharmaceutical products, from the process of its manufacture until validation of the final
product [5-7].

M. Mallah, et al. [8], recently reported the development of a transmission FTIR spectroscopic method
for the direct, low-cost and rapid quantification of paracetamol content in solid pharmaceutical
formulations.

In this study, a simple, rapid and reproducible methodology using (ATR) attenuated total reflectance
FTIR spectroscopy, quantum cascade laser spectroscopy and Raman spectroscopy for the direct
detection of acetaminophen in different pharmaceutical presentations were carried out. Statistics of
chemometrics routines such as hit quality index (HQI) values were applied to the obtained infrared
spectra. The results show that ATR-FTIR, QCL and Raman is useful for the detection of AAP in studied
drugs. Using the proposed method allows knowing if the drug is present as a desired product or if it is
counterfeit medicines.

2. Reagents, samples and sample preparation

The reagents used in this study correspond to standard and samples. As standard, pure analytical grade
acetaminophen purchased from Sigma-Aldrich, and was used to obtain the AAP reference ATR-FTIR
vibrational spectrum. As samples, some solid formulations in the form of tablets containing AAP as API
were used. These samples were obtained from the local markets of Cartagena-Colombia. The samples
acquired containing AAP were from different manufacturing laboratories, the laboratories were AG,
BEST, Genfar, Lafrancol, La Santé, MK, Sanofi-aventis and GRUNENTHAL.

Unlike other commonly used methods, in this method a simple preparation of the sample (tablets)
was used before the acquisition of the FTIR spectra. The only step involved is pulverize of a small
portion of the solid pharmaceutical samples (after removing the coating if it had) as a fine powder in a
mortar to reduce the particle size of the sample. We record 50 measurements (spectrum) for the eight
sample types and 5 independent measurements for the AAP standard, for a total of 55 mid-infrared
spectra with molecular information from AAP.

All samples and standards were analyzed using three systems of vibrational detection. Of these, two
using infrared spectroscopy and one using Raman spectroscopy.

First Infrared detection system: The spectra taken of AAP from the solid standard and commercial
tablets were recorded using the Thermo Nicolet 5700-FTIR spectrometer with ATR accessory and
Deuterated Triglycine Sulfate Detector (DTGS). The resolution was 4 cm−1 making an average of 64 scans
(scan) in the Mid IR region of 400-4000 cm−1.

Second Infrared detection system: The infrared vibrational spectra for the detection of AAP, were
carried out using a LaserScan ™ from Block Engineering, LLC, Marlborough, MA. These instruments
use an IR source of next generation quantum married laser (QCL) widely tunable. This type of sources
(laser) increases the sensitivity that are orders of magnitude greater than conventional spectroscopic
systems. The spectra Infrared were recorded in the spectral range of 1000-1600 cm−1, all spectra were
taken with 2 CoAditions and 4 cm−1 resolution.

Raman detection system: the instrument Renishaw InVia Raman Microscope. Objective, 20X;
Exposure time (s), 1s; laser, 785nm, 495 mW; Accumulation 1. Figure 1 shows the experimental
configuration used in this investigation.
Figure 1. IR spectral acquisition from commercial tablets of APP.

3. Results and Discussion

3.1. Obtaining vibrational spectra from AAP

Vibrational spectroscopy comprises NIR (780-2595 nm), mid-infrared (MIR; 400-4000 cm\(^{-1}\)), and Raman spectroscopy (Raman shift; 200-4000 cm\(^{-1}\)). The phenomenon of IR spectroscopy (NIR and MIR) is based on the absorption, reflection, and emission of the light from the sample, while Raman spectroscopy is based on the scattering phenomenon. However, both techniques deliver information about the fundamental vibrational and rotational modes of the molecules [9].

During IR spectroscopy, the sample spectrum is acquired by passing infrared radiation through the sample, which excites the molecular vibrations. The transmitted or reflected incident radiation from the sample is then analyzed by the detector. To obtain a Raman spectrum, the sample is illuminated by a monochromatic laser beam that interacts with the molecular vibrations. The scattered light from the sample is detected by the spectrometer [9].

Spectra of solid acetaminophen (AAP) Standard are shown in Figure 2. Spectra were taken using an attenuated total reflection (ATR) geometry by means of a Nicole 6700 FTIR spectrophotometer, see Figure 2-A; using a back reflection geometry by LaserScan, shown in Figure 2-B; using a Raman spectrometer as illustrated in Figure 2-C. To obtain the IR spectra of AAP, the air spectrum was taken as background. We can observe in Figure 2 that the spectra recorded, have the vibrational bands IR and Raman characteristics of AAP when they are compared with the FTIR/Raman spectra of the literature [10]. The intense lines of the benzene ring vibrations at 1606 and 1616 cm\(^{-1}\) for AAP were chosen as the characteristic peak in Raman.
Figure 2. Spectra of standard AAP using different vibrational instrumental techniques. 
A) ART-FTIR, B) QCL and C) Raman.
Figure 3. IR spectra representative of AAP from some commercial tablets using ART-FTIR.
Figure 4. IR spectra representative of AAP from some commercial tablets using QCL spectroscopy.

Figure 5. IR spectra representative of AAP from some commercial tablets using Raman spectroscopy.
3.2. Spectral analysis

The simplest spectral search is based on the calculation of the hit quality index (HQI) values [11]. The HQI is a numerical quantity that indicates the correlation between two spectra and has been widely used in spectroscopy to indicate the degree of spectral matching in library searches. HQI values can be calculated using various algorithms, but the two most commonly used are the Euclidean distance and spectral correlation algorithms. In the spectral correlation algorithm utilizes the Pearson product moment correlation coefficient, $r_{xy}$, which is a measure of the strength and direction of the linear relationship between two variables. This parameter is defined as the covariance of the variables divided by the product of their standard deviations. In this case, the spectral correlation algorithm is applied between two spectra: a reference (spectra form reference AAP) and an unknown spectrum to be identified (spectra form commercial tablets). Table 1 presents the spectral correlation coefficients for spectra form commercial tablets tested containing AAP.

It is generally acceptable to consider a spectrum of an unknown compound to be similar to one of the library when the spectral correlation coefficient is greater than ~ 0.85. Given this restriction, spectra measured from commercial tablets were correctly identified when ATR-FTIR coupled with spectral correlation algorithms were used.

Table 1. Values of spectral correlation coefficients (HQI) for spectra of AAP from various commercial tablets using ATR-FTIR.

|         | AAP St | AG    | BEST  | GENFAR | LA FRANCOL | LA SANTE | MK     | SANOFI | ZALDIAR |
|---------|--------|-------|-------|--------|------------|----------|--------|--------|---------|
| AAP St  | 1.00   | 0.998 | 0.997 | 0.993  | 0.645      | 0.998    | 0.999  | 0.952  | 0.993   |
| AG      | 0.998  | 1.00  | 0.999 | 0.994  | 0.649      | 0.999    | 1.000  | 0.960  | 0.996   |
| BEST    | 0.997  | 0.999 | 1.000 | 0.992  | 0.650      | 0.999    | 0.999  | 0.958  | 0.996   |
| GENFAR  | 0.993  | 0.994 | 0.992 | 1.000  | 0.665      | 0.997    | 0.994  | 0.976  | 0.993   |
| LA FRANCOL | 0.645  | 0.649 | 0.650 | 0.665  | 1.000      | 0.657    | 0.648  | 0.678  | 0.647   |
| LA SANTE | 0.998  | 0.999 | 0.999 | 0.997  | 0.657      | 1.000    | 0.999  | 0.967  | 0.997   |
| MK      | 0.999  | 1.000 | 0.999 | 0.994  | 0.648      | 0.999    | 1.000  | 0.956  | 0.995   |
| SANOFI  | 0.952  | 0.960 | 0.958 | 0.976  | 0.678      | 0.967    | 0.956  | 1.000  | 0.970   |
| ZALDIAR | 0.993  | 0.996 | 0.996 | 0.993  | 0.647      | 0.997    | 0.995  | 0.970  | 1.000   |

4. Conclusion

A system based on medium infrared spectroscopy using attenuated total reflection, QCL and Raman were used for the detection of acetaminophen as API present in commercial pharmaceutical tablets. Many vibrational characteristics of acetaminophen can be observed in the ATR, QCL and Raman spectra of analyzed tablets, when compared with standard AAP, so the vibrational contributions from the excipients is not a critical factor when deciding whether AAP is present in a certain tablet. In general, we have shown that ATR-FTIR spectroscopy is useful for the development of a rapid methodology for the detection and discrimination of AAP present in tablets when HQI is calculated.

Conflict of Interest.
The authors report there are no conflicts of interest.

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