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Direct assay and shelf-life monitoring of aspirin tablets using Raman spectroscopy

Chao Wang [a], Thomas J. Vickers [b], Charles K. Mann [b,*]

[a] *Athena Neurosciences, 800 Gateway Blvd. South, San Francisco, CA 94080, USA*
[b] *Department of Chemistry, Florida State University, Tallahassee, FL 32306-3006, USA*

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Abstract

A comparison was made between Raman and high-performance liquid chromatography (HPLC) analysis of aspirin tablets. The basis was an assay of aspirin content and the determination of salicylic acid produced by decomposition. Raman observations were performed directly on both intact and powdered tablet material. The limit of detection of HPLC with an ultraviolet detector is lower than that of the Raman measurement, but both are adequate for this application. The reproducibility of the Raman measurement is somewhat better than that of the HPLC measurement. Both methods were used in a degradation study in which samples were stored in a humid atmosphere for a maximum period of 8 weeks. Aside from somewhat higher salicylic acid responses from the HPLC method, which were attributed to hydrolysis during chromatography, results from the two methods were comparable. Direct Raman measurements are faster and do not require the use of solvents. © 1997 Elsevier Science B.V.

Keywords: Aspirin; Direct analysis; Tablets

1. Introduction

A Raman spectroscopic measurement is made by illuminating an object with a narrow band source and examining the light that is scattered by it. The scattered radiation consists of three components, an elastically scattered (Rayleigh) line which is bracketed by two inelastically scattered side bands. The vibrational spectrum of the target is carried by both side bands; however, the low-frequency (Stokes-shifted) band is the stronger and was used in all measurements reported. Raman spectroscopy, because it is a scattering measurement, is particularly suitable for direct analysis of solid samples. This is a report on its application to the assay of aspirin tablets and to the measurement of the extent of their degradation in storage. We have chosen the aspirin assay because aspirin is a well characterized material and because of the accessibility of comparison methods for its assay. The demonstrated success of the Raman method for the aspirin assay may suggest its utility for direct analysis of other tablets and powders.

* Corresponding author. Tel.: +1 904 6443747; fax: +1 904 6448275; e-mail: mann@chem.fsu.edu.

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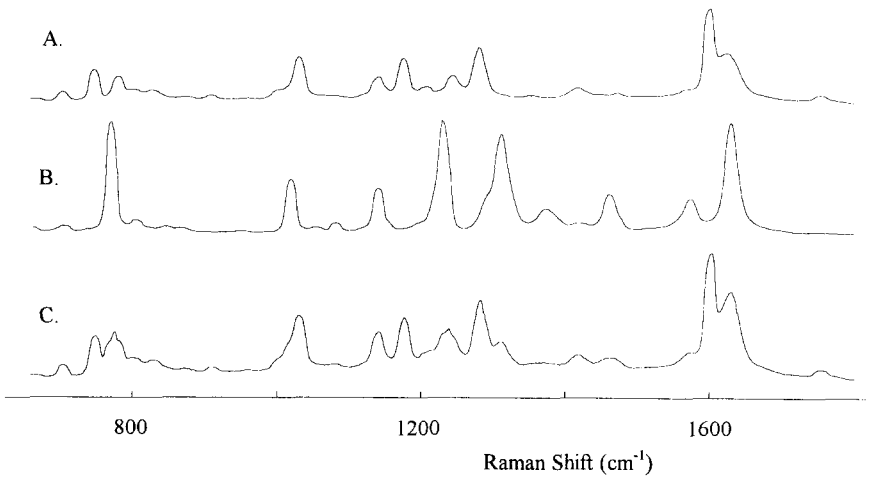

Fig. 1. (A) Aspirin; (B) salicylic acid; (C) aspirin sample that had been exposed to humid air for 8 weeks. These are powdered samples, prepared under the conditions of the quantitative analysis. Spectra were recorded at a resolution of about 15 cm^{-1}.

The stability of aspirin in tablet and solution form has been examined [1–6]. Aspirin tablets are sensitive to moisture and gradually decompose through hydrolysis to form salicylic and acetic acids, the latter of which is lost due to its volatility [7]. Analytical methods used in the determination of the active ingredient of aspirin tablets as well as of the residual degradation product, salicylic acid, involve colorimetric methods [8–13], thin-layer chromatography [14,15], titrimetry [16–18], ultraviolet spectrophotometry [19–22], fluorimetry [23–26], second-derivative spectroscopy [27,28], voltammetry [29], gas–liquid chromatography [30], normal-phase high-performance liquid chromatography (HPLC) [31–33], and reverse-phase HPLC [34–40].

Because of its high sensitivity and short finish time, the reverse-phase HPLC method is widely used. However, there are disadvantages. Degradation during sample preparation and analysis has been observed to cause false high results for the hydrolysis products [35]. In addition, samples must be brought into solution. This introduces a solvent disposal problem which is increasingly burdensome. The process of dissolution or extraction makes the analysis time-consuming and can be a source of uncertainty.

We have established conditions for direct analysis of solid tablet formulations for aspirin and salicylic acid by Raman spectroscopy. This procedure has been applied to the analysis of aspirin tablets that have been exposed to humid atmosphere. To assess the validity of the results, the same samples have been analyzed using reverse-phase HPLC.

2. Experimental

2.1. Reagents

The salicylic acid and starch used were ACS reagent grade (Aldrich). The aspirin was laboratory grade (Aldrich). HPLC grade acetonitrile (Fisher Scientific) was used in the chromatographic work. All other chemicals were of reagent grade and were purchased from Fisher Scientific.

2.2. Apparatus

Quantitative Raman measurements were made with a filter/single spectrographic system. The filter was a holographic Raman notch filter from Kaiser Optical Systems. The spectrograph was a Jobin-Yvon HR640 (640-focal length) equipped with a 600-groove mm^{-1} grating and operated with a 200-μm slit width. The multichannel array detector was a 298 × 1152-pixel front-illuminated

CCD from Princeton Instruments. All 298 pixels are binned in the direction parallel to slit height. A 600-groove mm^{-1} grating provides a spectral window for simultaneous measurement with a width of about 1800 wavenumbers. The laser was a Spectra-Physics model 2017 5-W argon ion operated at 514.5 nm. The fiber-optic probe was locally constructed in the six-around-one configuration. There is no filtering in the probe to reject the Raman signal of silica produced by the fibers. The matching optics between probe and spectrograph produce approximately 3-fold magnification of the fiber image.

For survey spectra, a Kaiser HoloProbe dispersive system was used. This instrument consists of an axial transmissive *f*/1.8 spectrograph fitted with a grating (HoloPlex™, Kaiser Optical Systems) that disperses light into two segments that are simultaneously imaged on a CCD detector. This provides a spectral range of approximately -50 to 4500 cm^{-1} Raman shift relative to a 532-nm laser at a resolution of about 5 cm^{-1}. Excitation is from a 50-mW solid-state frequency doubled Nd:YAG laser, which generates an excitation radiation of 532 nm. The detector is an electrothermally cooled MPP-type 298×1152 front-illuminated CCD camera from Princeton Instruments (Princeton, NJ). The system is coupled to the sample through a fiber-optic probe that is fitted with filters to remove extraneous light scattered by the sample. This consists of the Rayleigh line and the Raman signal produced by the silica of the illuminating fiber.

Chromatography was done on an instrument that consisted of a Beckman Model 114M pump, a manual injector with a 20-μl sample loop and a Beckman Model 168 diode array detector. It was under computer control using the Beckman Gold software through the Beckman Model 406 analog interface. Separations were made on a 150×4.6 mm column that was packed with 120-Å and 5-μm BDS Hypercil C_{18} stationary phase. The mobile phase was 50:50 water–acetonitrile with 0.1% (v/v) trifluoroacetic acid. The system was operated with an ultraviolet detector at 285 nm, a flow rate of 1.2 ml min^{-1}, and ambient temperature.

2.3. Spectroscopic measurements

2.3.1. Measurement conditions and data treatment

The J.-Y. spectrometer was operated with 200-μm slits to produce a resolution of about 15 wavenumbers. Laser power at the sample was approximately 75 mW. Measurement conditions were arranged to have on-chip integration produce approximately half of full scale response for the strongest feature in a spectrum. Effective exposure times were established by ensemble averaging replicates of the basic exposure. The results reported below typically reflect averaging 76 1-s exposures.

All spectra were corrected for instrument response in a two-stage process. In the first stage, a blank representing the dark response of the detector is subtracted. Then wavelength-dependent spectrometer behavior was corrected by ratioing measured spectra with that of a white light source.

Analytical results were obtained by doing least-squares fits [41] of the 1530–1730 cm^{-1} range of reference spectra to the corresponding spectral range of experimental spectra. In Fig. 1, the references for aspirin and salicylic acid are shown, together with the spectrum of the sample that was exposed to moist atmosphere for 8 weeks. The fitting range includes a ring mode of aspirin at about 1605 cm^{-1} and a ring mode of salicylic acid at 1634 cm^{-1}. In addition to the reference sets shown, two additional arrays were included in the fitting operation. One contained a DC level and the other a ramp function. These compensate for any offset or sloping baseline in the experimental data.

2.3.2. Sampling

The purpose of the work was to make measurements on tablets that are a 90:10 (w/w) mixture of aspirin and starch. The details of the sampling jig that was used have been described elsewhere [42]. It consisted of a cylindrical glass-bottomed container. The laser probe was mounted to give a 5-mm spacing between the probe tip and the sample window. The sample container was rotated at about 1.6 rpm to average the sample response.

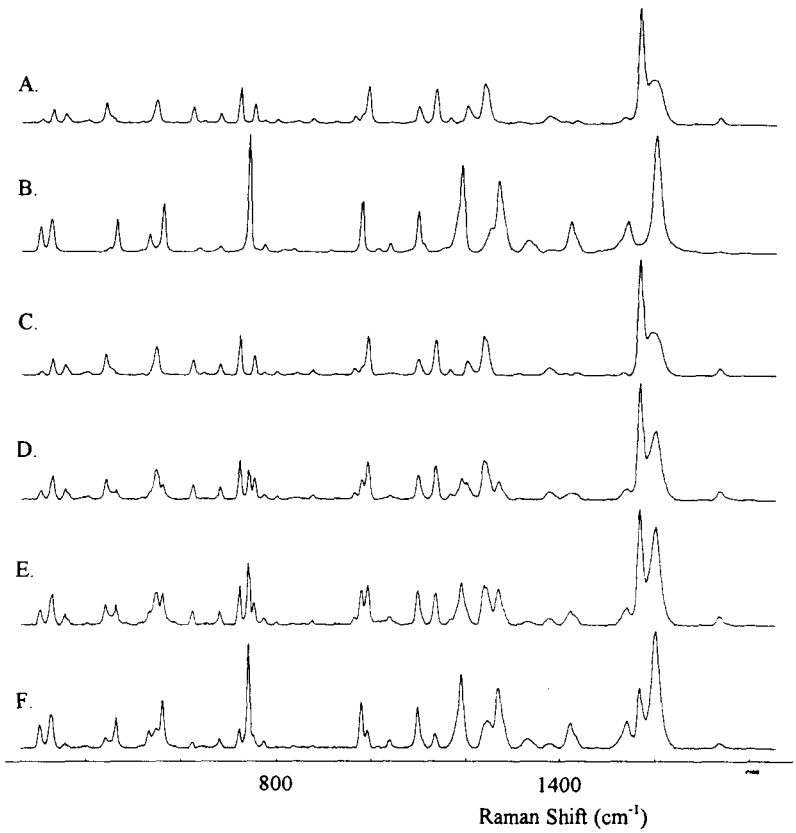

Fig. 2. (A) Aspirin; (B) salicylic acid; (C) aspirin tablet as received from the supplier; (D) aspirin tablet after exposure to humid air for 2 weeks; (E) tablet after exposure for 4 weeks; (F) tablet after exposure for 8 weeks. These are direct measurements on intact tablets, made at a resolution of about 5 cm^{-1}.

Raman peak shapes are unaffected by the variations in particle size that are encountered in this work. However, light collection efficiency is affected by particle size, and some control of sample packing is necessary for quantitative work. Tablets were gently ground by hand and then homogenized with a vortex mixer. The powder was poured into the sample container and gently tamped using a hand-held metal plunger. The reproducibility of this procedure was examined. The critical step is homogenization by mixing. Approximately 8 min of homogenization were required to produce a relative standard deviation of 0.2% in measurement of five different areas of a sample.

In the work described below, samples were first analyzed by the Raman method and then were dissolved and analyzed by the HPLC method.

2.3.3. Calibration

The laser, detector and optical geometrical arrangements are sufficiently stable that it is not necessary to use internal standards to compensate for variations during the course of a single measurement session. It is necessary to make some provision to assure that measurements from different sessions are placed on a common basis. It is also necessary to calibrate the collection efficiency of the sampling arrangement in order to be able to express results in quantitative terms.

Inter-session calibration can be done by recording the response of a fiduciary sample and rescaling all data by the factor that is needed to bring the reference set to its standard value. However, in this work, inter-session calibration was avoided by running a standard curve at each session.

Table 1
Results of quantitative analysis in accelerated degradation study[a]

Storage time (weeks)	Raman analysis[b]			HPLC analysis		
	Fraction of aspirin recovered as:		Mass balance	Fraction of aspirin recovered as:		Mass balance
	Aspirin	Salicylic acid		Aspirin	Salicylic acid	
0	99.65	0.01	99.66	98.90	0.77	99.67
	100.18	0.00	100.18	99.19	0.81	100.01
	99.06	0.00	99.06	100.42	0.85	101.27
2	96.19	4.14	100.32	96.46	4.75	101.21
	95.95	4.16	100.11	95.29	4.68	99.97
	95.97	4.24	100.21	95.98	4.74	100.72
4	94.66	6.51	101.17	94.47	6.58	101.05
	96.53	6.59	100.12	94.62	6.57	101.19
	93.93	6.58	100.52	93.49	6.55	100.04
8	89.87	10.08	99.95	91.26	9.67	100.93
	91.44	9.90	101.34	91.46	9.70	101.16
	91.04	10.22	101.26	91.63	9.64	101.26

[a] Both Raman and HPLC measurements were done on the same samples, produced by grinding 20 tablets for each reported value.
[b] Exposure time of 76-s.

2.3.4. Standard curves

The samples used to generate standard curves were produced by binary addition. The aspirin curve spanned the range 50–100% and was made by successive additions of equal weights of pure aspirin starting with a 50% mixture of aspirin and starch to produce five concentration levels. The salicylic acid curve covered the range 0–25%. It was made by successive additions of equal weights of starch starting with a 50% mixture of salicylic acid and starch to produce seven concentration levels.

The calibration curve for aspirin was determined to have a slope (S) of 0.0082, an intercept (I) of 0.1761, standard deviation of slope (σ_{sl}) of 8.5×10^{-5}, standard deviation of intercept (σ_{int}) of 0.0071 and correlation coefficient (CC) of 0.9998. A second determination on separately prepared samples gave $S = 0.0088$, $I = 0.126$, $\sigma_{sl} = 2.1 \times 10^{-4}$, $\sigma_{int} = 0.017$ and CC = 0.9992. This difference was mainly caused by differing responses by the 50% samples of the two trials. We attribute it to failure to achieve adequate homogeneity in the 50% sample of the second trial. The other samples of that set are in excellent agreement with their counterparts of the first set.

The limits of detection (LOD), defined as $3 \times \sigma_{int}/S$, were 0.36 and 0.51% for the two trials. For values given just above, the ordinate has been normalized to a least-squares fit of unity for pure aspirin. However, the more relevant measure of precision is the standard deviation of the response for the aspirin concentration used. For the 50% aspirin sample, the relative standard deviation was 0.15%, based on six replicates.

For salicylic acid, the corresponding values were $S = 0.013$, $I = 0.0021$, $\sigma_{sl} = 1.3 \times 10^{-4}$, $\sigma_{int} = 0.0014$, CC = 0.9998, LOD = 0.33%. These are normalized to give a least-squares fit of unity for pure salicylic acid. As is frequently true for analyses of mixtures, the limits of detection are controlled by the effectiveness of sample preparation, rather than by the intrinsic sensitivity of the measurement. The actual response for each of the analytes is strong enough to produce responses with S/N larger than 3 at the quoted LOD concentrations. The relative standard deviation for the lowest salicylic acid concentration was 0.07%, based on six replicates.

2.4. HPLC measurements

Samples that had been analyzed by the Raman method were dissolved in a 92:8:0.5 vol.% mixture of acetonitrile–methanol–85% phosphoric acid [40]. Before injection, samples were filtered through 0.45-μm nylon filters. The recovery from filtering was estimated for six samples by comparing the peak areas measured on samples before and after filtering. For aspirin it was 99.80% with relative standard deviation of 0.84%. For salicylic acid it was 100.15% with 1.23% relative standard deviation. The mobile phase was 50:50 water–acetonitrile with 0.1% trifluoroacetic acid.

The reproducibility of the measurements was evaluated by determining the precision of retention times and of peak areas for a sequence of six 20-μl injections of working standard. For 0.033% aspirin, the standard deviation of retention time (σ_t) was 0.29%. The standard deviation of area (σ_a) was 0.24%, based on three replicates. For 0.0025% of salicylic acid, σ_t was 0.50% and σ_a was 0.18%, based on three replicates.

2.4.1. Standard curves

Working standards for aspirin were prepared in triplicate at five concentration levels in the range 0.02–0.033%. Data for the analytical curves were: $S = 273$, $I = 0.26$, $\sigma_{sl} = 1.16$, $\sigma_{int} = 0.20$, CC = 0.99997, LOD = 0.00022%. Here the ordinate values are arbitrary detector responses. The LOD definition is the same as that used for the Raman measurements. For salicylic acid the corresponding values for the concentration range 0.00016–0.0025% are: $S = 722$, $I = -0.15$, $\sigma_{sl} = 5.14$, $\sigma_{int} = 0.67$, CC = 0.99992, LOD = 0.000028%.

3. Results and discussion

3.1. Accelerated tablet degradation

Four sets of 60 aspirin tablets, weighing 365.2 ± 3.6 mg each, were used in this study. The nominal composition was 325 mg of aspirin and 40 mg of starch. The samples were exposed to moist atmosphere by storing them in a desiccator over water at 60–65°C. The sets were held for 0, 2, 4 and 8 weeks.

The results are shown in the survey spectra in Fig. 2. Curves A and B show sections of reference spectra for aspirin and salicylic acid. Curves C–F are the corresponding spectral regions for a tablet taken from each of the four groups at the end of their exposures. These are spectra that were made of the surface layer of intact tablets. For each condition, one measurement was made on a single tablet. The increase in salicylic acid on the surface of the tablets as exposure time increased is very evident.

3.2. Quantitative results

For purposes of quantitation, the assumption was made that the hydrolytic formation of salicylic acid from aspirin is the only significant process involved. The initial composition of the tablets is taken as 89.0% aspirin and 11.0% starch. For each stage, samples were ground and subjected to Raman analysis. Then the material was dissolved and analyzed by HPLC. To handle the samples with the mixing apparatus that was used, each of the four groups of samples were subdivided into three groups of 20 tablets for analysis. Based on the assumptions given above, the aspirin equivalent of the salicylic acid formed was calculated and a mass balance established. The results shown in Table 1 represent averages of 76 1-s exposures.

The agreement between the Raman and HPLC results is generally good except for the zero storage time set corresponding to the lowest percent salicylic acid. We believe this difference is attributable to aspirin hydrolysis occurring during the HPLC determination.

4. Conclusion

We have demonstrated the feasibility of direct Raman-based determination as a basis for assaying aspirin and for analysis of the major degradation product, salicylic acid. The advantage of a direct Raman measurement stems in part from reduction in the number of sample handling steps that must be performed. Each of these imposes a cost in terms of time and in terms of uncertainty

in the result. The occurrence of aspirin hydrolysis during the HPLC determination clearly points out the advantage of direct determination. The spectroscopic analysis has the additional advantage of not requiring the use of any solvents.

The absolute limit of detection for the HPLC procedure is lower than that of the Raman measurement. However, both are sufficiently sensitive for the purpose, and the relative uncertainties of the two measurements are rather similar. Taken at the lower end of the calibration curves, the estimate of relative standard deviation for the Raman measurement of aspirin was 0.15%, based on six replicates. The corresponding value for the HPLC measurement was 0.50%, based on three replicates.

Following the procedures outlined above, the Raman analysis requires about 15 min compared with around 90 min for the HPLC analysis. However, of that time, approximately 13 min of the Raman analysis was used for grinding and mixing the sample. This was done in order to place the results from the two types of measurements on the same basis. In a quality control application, it would be possible to develop correlations between surface observations and those done on homogenized samples, which would make it possible to forgo sample treatment in the Raman measurement. Dyed tablet coatings would not interfere with these measurements. Pigmented tablet coatings would raise the LOD of the analysis.

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