# Determination of acetylsalicylic acid in aspirin using Total Fluorescence Spectroscopy

## Application Note


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## Introduction

Fluorescence is the emission of light by a molecule in an excited electronic state. The phenomenon of fluorescence in most cases represents the energy emitted when a substance returns from an excited or higher energy state to its normal or lower energy state.

Two types of information may be obtained when the fluorescence emission is studied:

1. The intensity-wavelength distribution, which is an indication of the electronic structures of the molecule.
2. The intensity at any wavelength where emission occurs which is an indication of the concentration of the fluorescent substance in the solution.

It is this second aspect of fluorescence that we are mainly concerned with when using the total fluorescence method of analysis.

In the total fluorescence measurement, the sample is irradiated with radiation of a known wavelength and the fluorescence emission which occurs over the complete wavelength range is measured.

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The emission is measured at 90° to the excitation direction to minimize scattering effects as shown in Figure 1. The amount of radiation from the fluorescent substance in the sample is then used as an indication of the sample concentration [1].

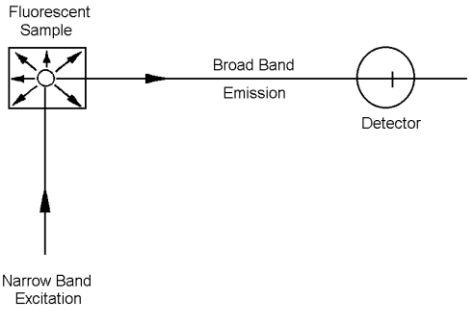


**Figure 1.** Emission measurement at 90° to the excitation direction to minimize scattering effects

This at work describes one of the many pharmaceutical applications of fluorescence spectroscopy. Compounds with an intrinsic fluorescence that normally appear in drug preparations can be easily analyzed for conformance with label specifications. Fluorometric determination of acetylsalicylic acid (ASA) as found in aspirin tablets, can be performed in 1% acetic acid v/v in chloroform solvent. In this solvent the fluorescence excitation wavelength for ASA is approximately 290 nm, and the emission band approximately 300-420 nm.

## Equipment

- Cary 1/3E UV-Vis spectrophotometer
- Cary 1/3E Fluorescence accessory
- Secondary (emission) filter UG5
- Stoppered quartz fluorescence cells
- Filter paper (Whatman, #1 qualitative)
- Cary 1/3E Concentration Application software
- Printer

### Reagents

- Chloroform, Spectroscopy grade (BDH)
- Acetic acid, Reagent grade (BDH)
- Acetylsalicylic acid (ASA), pure compound (AJAX Chemicals)
- Aspirin tablets

### Standard preparation

10 mg of pure acetylsalicylic acid was prepared in a 100 ml standard flask with 1% acetic acid v/v in chloroform and diluted to appropriate concentrations with the same solvent.

ASA Standards: 0.0, 2.0, 4.0, 6.0, 15.0, 50.0 mg/L.

A calibration curve for acetylsalicylic acid was then obtained using the Concentration Application software.

**Note:** Use the maximum SBW when optimizing the parameters and adjust the gain so that the highest standard reads the maximum %T, this will ensure that you obtain the maximum fluorescence intensity for each of your standards.

### Sample preparation

The aspirin tablets for the sample analysis were crushed and dissolved using the 1% acetic acid/ chloroform solution, and made up to volume in a 100 ml standard flask. The solution was then rapidly filtered through filter paper. Dilutions of 1 to 100, and then 1 to 1000 were performed. The fluorescence intensity for each sample was compared with a standard of acetylsalicylic acid using the calibration curve obtained previously.

**Note:** For accurate analysis, fluorescence measurements of the standards and sample aspirin tablet should be taken within 45 minutes of dissolving the solid, thereby avoiding significant hydrolysis of ASA to salicylic acid[2].

## Results and discussion

Fluorescence intensity is proportional to the number of fluorescing molecules in the sample, which would assume that a linear relationship exists between fluorescence intensity and concentration of the sample. In practice however, this linearity is often absent and curvature is observed. A process called 'self absorption' is largely responsible for this curvature which occurs when the excitation and emission spectral envelopes overlap to a small extent. When this happens some of the emitted radiation is able to re-excite ground state molecules and is lost. The amount of self absorption increases with increasing concentration. Figure 2 shows how the emission intensity increases and decreases in a typical fluorescence calibration graph[1]. This phenomenon can also be seen in the acetylsalicylic acid calibration curve shown in Figure 3.

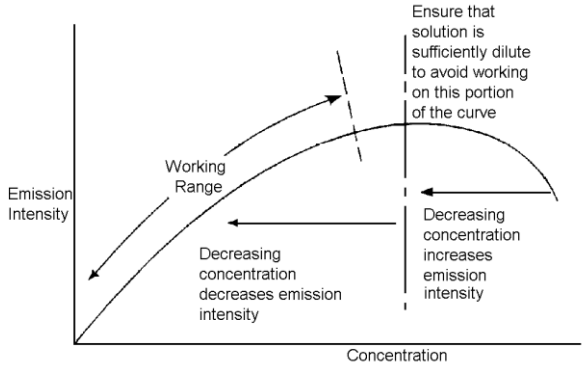


**Figure 2.** Emission intensity in a typical fluorescence calibration graph

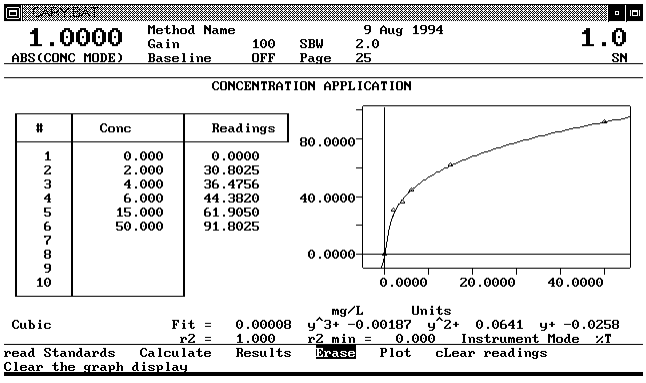


**Figure 3.** Emission intensity in the acetylsalicylic acid calibration curve

**Table 1.** Results for acetylsalicylic acid in 1% acetic acid/chloroform solution

| Standard | Conc (mg/L) | Emission |
|---|---|---|
| 1 | 0.0 | 0.00000 |
| 2 | 2.0 | 30.8025 |
| 3 | 4.0 | 36.4756 |
| 4 | 6.0 | 44.3820 |
| 5 | 15.0 | 61.9050 |
| 6 | 50.0 | 91.8025 |

**Table 2.** The level of acetylsalicylic acid found in the samples

| Sample # | Level of ASA (mg) |
|---|---|
| 1 | 309 |
| 2 | 307 |
| 3 | 298 |
| 4 | 302 |
| 5 | 304 |

According to the manufacturer's specifications, the commercially available aspirin tablets contained 300 mg aspirin. For the tablets to comply with the requirements of the British Pharmacopeia, the content of aspirin needed to be between 95% and 105% of the prescribed or stated amount[3].The results obtained indicate that all the tablets meet the requirements.

## Conclusion

The Cary 1/3E Fluorescence accessory together with the Cary Concentration Application software can be used to successfully determine the amount of acetylsalicylic acid in a commercially available aspirin tablet.

## References


1. Lane, R.J, UV-Visible Spectroscopy - Applications of Total Fluorescence. **(1973).**
2. Schenk, G.H. and Boyer, F.H., Miles, C.I, and Wirz, D.R. Analytical Chemistry **44**(9), **(1972),** pp 1593-1598.
3. Department of Health, British Pharmacopeia, Volume **2**, **(1993).**

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