Determination of Aspirin Tablet Manufacturers by an NMR-based Metabolomic Approach

Moon-Young Choi‡, Sunmi Kang‡, Jeong Hill Park1 and Sung Won Kwon†

1College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Korea
2Department of Biochemistry and Center for Advanced Medical Education by BK21 project School of Medicine, Inha University, Incheon 400-712, Korea
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ABSTRACT – Aspirin or acetylsalicylic acid, a member of the salicylate family, is frequently used as an analgesic, antipyretic, anti-inflammatory and antiplatelet drug. Because aspirin is chemically unstable in water and heat for tablet formulation, additives including lubricants are used in preparing aspirin tablets, using a dry-granulation process. Aspirin tablets are produced by a number of manufacturers which usually use their own unique combination of additives during the manufacturing process. In this study, we employed an NMR based metabolomics technique to identify the manufacturers of various aspirin tablets. Aspirin tablets from six different companies were analyzed by 1H 400 MHz NMR. The acquired data was then integrated and processed by principal component analysis (PCA). Based on the NMR data, we were able to identify peaks corresponding to acetylsalicylic acid in all of the six samples, whereas different NMR patterns were found in the aromatic and aliphatic regions depending on the unique additive used. These observations led to the conclusion that the differences in the NMR patterns among the different aspirin tablets were due to the presence of additives.

Key words – Aspirin, Additives, Metabolomics, NMR, Principal components analysis (PCA)

A tablet is a pressed solid form of a drug that contains both active components and additives. Methods used for the manufacture of tablets include granulation, direct compression and semi-direct compression methods. In order for active substances to be formulated into tablets, they must possess several physical properties such as mobility, coherence, lubrication and the ability to disintegrate, which are difficult to meet for most active substances. Therefore appropriate additives are added to tablets to improve strength, disintegration, rate of release, stability, etc. Pharmaceutical companies use different types and amounts of drug additives and this aspect of the manufacturing process is frequently patent-protected.

Aspirin or acetylsalicylic acid is a synthetic drug with analgesic, antipyretic, anti-inflammatory and antiplatelet activities.1) The analgesic and anti-inflammatory effects of aspirin are due to the fact that it is a cyclooxygenase (COX) inhibitor. Although aspirin has long been used as an antiplatelet drug, the use of a low dose of aspirin has received spotlight these days, because of its preventive effect on arteriosclerosis. Numerous pharmaceutical companies produce aspirin for different therapeutic purposes using their own unique manufacturing processes. Aspirin tablets are produced by the dry-granulation method, in which lubricants are used because aspirin has a very low tolerance to water and heat. Frequently used lubricants include stearic acid, magnesium stearate, calcium stearate, polytetra fluoroethylene (PTFE), liquid paraffin, vegetable oils, waxes, etc.2)

Metabolomics has emerged as an efficient tool in a number of areas including the synthetic, cosmetic and food industries as well as pharmaceutics because it has the capability of providing a high-throughput analysis and targeted quantitative/ qualitative measurements, which can be useful in monitoring the quality of a sample. It also has merits, in that statistics algorithms can be employed to identify unknown markers among samples.3–8)

Although acetylsalicylic acid is the major active component of aspirin tablets, they also contain a variety of minor inactive components, i.e., additives depending on the manufacturer. This fact prompted us to examine the components of aspirin tablets as a tool that would permit different manufacturers of such tablets to be distinguished. For this purpose, we used an NMR-based metabolic approach, which includes collecting data by gas chromatography (GC) and nuclear magnetic resonance (NMR), converting the data by means of a statistics algorithm (binning and alignment), performing a multivariate statistical analysis using a principal components analysis (PCA) and then interpreting the chemometric results. As a result, we succeeded in distinguishing a few groups of tablets.

†본 논문에 관한 문의는 이 저자에게요
Tel : 02)880-7844, E-mail : swkwon@snu.ac.kr
‡These authors contributed equally to this work.
Figure 1—The gas chromatograms of the 6 aspirin samples.
out of six different manufacturers.

**Experimental**

**Materials**

Camphor, CD$_3$OD, D$_2$O, NaH$_2$PO$_4$ anhydrous and Na$_2$HPO$_4$ anhydrous were purchased from Sigma (St. Louis, MO, USA). Tetradeutered trimethylsilanepropionic acid (TSP) was purchased from Merck (Darmstadt, Germany).

**Aspirin samples**

Aspirin tablets from six pharmaceutical companies (A, B, C, D, E and F) were collected for analysis. Each tablet was powdered and stored in vacuum packages until analyzed. Ten tablets from each company were used to prepare the NMR samples (total sample number for six companies, 60).

**Sample preparation for GC-FID**

Powdered samples of 10 mg from each aspirin were dissolved in 1 mL of methanol and 100 µL of a 50 mg/mL solution of camphor was spiked as an internal standard. The resulting samples were then analyzed by GC-FID.

**GC-FID**

GC analyses were performed using a Hewlett-Packard 6890 series system (Palo Alto, CA, USA) connected to a flame ionization detector (FID). A Hewlett-Packard HP-FFAP fused silica capillary column (i.d. 0.2 mm, length 50 m, film thickness 0.33 µm; Palo Alto, CA, USA) with a helium carrier gas as a linear velocity of 24 cm/s were used for small molecule profiling. The following conditions were used for the operation: an injection system split ratio of 50, injection temperature of 250°C, isothermal at 200°C for 30 minutes and a flame-ionization detector temperature of 280°C.

**Sample preparation for NMR**

Each sample of 100 mg was extracted with CD$_3$OD and D$_2$O mixture (1:1) that contained 10 mM sodium phosphate. The extraction was carried out by sonication at room temperature for 20 minutes in a bath-type sonicator. Tetradeutered trimethylsilanepropionic acid (TSP, final concentration of 0.025%) was added as an internal chemical shift reference. Insoluble materials were removed by centrifugation at 13,000 g for 1 minute and the supernatant was transferred to a standard 5 mm NMR tube. One dimensional NMR spectra were obtained on a 400 MHz NMR (Bruker, Germany).

**Data analysis**

Data was processed with an in-house developed program on a Linux workstation. The water region (4.6~5.8 ppm) was excluded from the raw data for analysis. Peak intensities of the spectra were integrated at every 0.04 ppm using an in-house constructed Perl program. The output file was imported into the SIMCA-P software for the PCA (Umetrics, Sweden).

**Results and Discussion**

In preliminary experiments using GC-FID, we found that some of the GC chromatograms of aspirin tablets showed different chromatographic patterns. Figure 1 shows the GC chromatograms for the six aspirin tablet samples that had been manufactured by six different pharmaceutical companies (A, B, C, D, E and F). Because a comparison of the chromatograms...
grams in chromatographic methods is usually not as precise as that for the chemical shift values in NMR, chromatographic data need a complicated sliding window format rather than a simple binning process for converting data to the statistics algorithm compared to NMR method, which is why NMR is more widely used in metabolomic experiments. Therefore we used the NMR-based method as the main tool in this study while GC was used as an auxiliary tool for monitoring different data patterns.

To further investigate the different patterns obtained in the GC-FID runs, we analyzed six different types of aspirin tablets (ten samples from each company) by $^1$H 400 MHz NMR. From the NMR experiments, we noted differences in the NMR patterns in the aromatic and aliphatic regions while all of the acetylsalicylic acid peaks from all sixty samples were present at the same chemical shifts (Figure 2). All of the raw NMR data for the sixty samples were converted and treated by PCA using the SIMCA-P program. The majority of the samples could be segregated into two groups and most of samples originated from the same company were classified to the same category. However, some of the components were found in both groups in the score plot, leading to an incomplete grouping by PCA (Figure 3A). When the loading plot was examined, no unique ppm region could be found that divides these two groups (Figure 3B). Therefore, PCA using one pair out of six by combination was performed instead and it was found to be successful for some pairs. In the pair A and C, the NMR data were divided into two parts (Figure 4A) and the discriminating ppm region was determined to be in the aromatic region (Figure 4B). Similarly, the NMR data for pair E and F showed two

![Figure 3](image-url)
parts (Figure 5A) with a discriminating ppm region at around 7 ppm in the aromatic region (Figure 5B). The rest of pairs were not successfully distinguished (data not shown). In principle, since PCA reduces variables by binding variables with similar values, the discriminating power is decreased if the variables of similar value have a larger value than variables with relatively different values. In this study, the samples showed a relatively poor separation by PCA. This can be explained by the native property of PCA, i.e., the analyzed samples all contained acetylsalicylic acid as the major component whereas additives such as lubricants were present as minor components in the tablets, thus decreasing the discriminating power. This suggests that PCA applications may not be suitable for a metabolomic approach to discover low abundant signals that differ slightly among similar NMR data. It is likely that the above pairs that were successfully distinguished contained very different types of additives.

**Conclusion**

A metabolomic pattern recognition algorithm was applied using NMR based metabolomic approach to discriminate between various manufacturers of aspirin tablets. For this purpose we carried out some preliminary GC-FID experiments and noted differences in the chromatographic patterns. Then we performed $^1H$ NMR measurements, from which we were able to note differences in NMR patterns in the aromatic and aliphatic regions that were due to the additives contained by...
Our NMR-based metabolomic approach was found to be partially successful in discriminating between manufacturers of aspirin tablets. This is because the contents of the major components were higher than the minor inactive components (additives such as lubricants), as evidenced by NMR spectral data. As a result, the dividing effects were hidden. In order to circumvent these problems, advanced statistical approaches need to be developed and more diverse datasets from various instrumental analyses should be collected.

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