

Atomic Absorption Analysis of Toxic Heavy Metal Impurities in Various Commercial Aspirin Formulations

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Abstract: Aspirin is the most extensively used mild analgesic and antipyretic drug. In Libya as well as worldwide, aspirin is purchased without prescription in many forms and is imported into the country without any control. However, conditions for packing, storing and preventing the drug from damage are not properly followed. There are no supervision or quality control procedures on the validity and chemical composition of the drug. In this study, thirteen available aspirin forms imported from different countries and one sample from local factory were collected and analyzed for comparison. The quality of the samples was examined in terms of active ingredient (acetyl salicylic acid- ASA), toxic heavy metal and salicylic acid impurities. ASA contents were analyzed using volumetric titration and HPLC method. The toxic heavy metal impurities were determined using atomic absorption spectrophotometry, and salicylic acid impurity was determined by fluorimetric method. The active ASA contents were found in the range from 87 to 104%. Comparison of the determined ASA contents with the actual contents per tablet indicated that only 14% of the samples were in identical values, whereas; 57.1% were exceeded and 28.6% were less than the actual content per tablet. The salicylic acid impurities were under permissible limit. Although, the results for the toxic heavy metals impurities showed significant variations among the samples, but all were under the limit permitted by the world health organization.

Keywords: Aspirin, Salicylic Acid, Acetylsalicylic Acid, Toxic Heavy Metal, Atomic Absorption Spectroscopy, HPLC

1. Introduction

Aspirin (acetylsalicylic acid) is one of the most widely used minor analgesics. It is used to relieve minor aches and pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication (Cheng, 2007, Aukerman et al. 2000, Jeffreys 2005, Reynolds 1982, Krumholz et al, 1995, Tang et al, 2012). Long-term therapy with low dose of aspirin has also been used to prevent heart attacks, strokes, and blood clot formation in people at high risk (Brambilla et al, 2010, Smith et al, 2012). It has also been suggested that low doses of aspirin may be given immediately after acute myocardial infarction to reduce the risk of re-infarction (Krumholz et al, 1995, Borzak et al, 1998, Marcus & Broekman 1998, American Heart Association 2008). There are also some side effects associated with the regular use of aspirin such as lung

cancer, colon cancer, gastrointestinal bleeding etc. (Moysich et al., 2002. Temple 1981, Endo et al. 2014)

The purity and homogeneity of aspirin preparations has been debated over several years. The main concerns regarding the aspirin preparations have been active ingredients and the impurities of salicylic acid and toxic heavy metals. One study, analyzing active ASA ingredients in several aspirin brands, found that ~5% tested samples were failed to meet the USP XIX limits for active ASA contents and ~10% failed to meet the limits for salicylic acid impurity (Juhl & Kirchhoefer, 1980). Another study found that some of the aspirin formulations contained salicylic acid impurity exceeding the USP 1980 limit of 0.3% salicylic acid per tablet (Salako et al., 1989).

Frenata *et al.* (2002) and Rodrigues *et al.* (2004) have determined active acetylsalicylic acid (ASA) contents in various aspirin samples, and the results obtained were comparable to the results obtained by pharmacopeia titration method and HPLC method. Yang *et al.* (2004) have also analyzed active ASA ingredients in various aspirin brands and found that some aspirin brands may contain up to 110% of active ASA contents. Miyoshi & Saiki (2009) and Iskandar *et al.* (1986) have analyzed various trace elements by using 'instrumental neutron activity analysis (INAA)' of trace element in various aspirin samples. The results obtained by these investigators were comparable with the certified standard reference materials for various trace elements as reported in The International Pharmacopeia (WHO, 2011). In another study, using a quantitative HPLC method determined salicylic acid impurities in bulk aspirin and plane buffered aspirin tablets (Kirchhoefer & Juhl, 1980). They found that excipients and impurities did not interfere with the quantification and the recovery was 100% and salicylic acid impurities were within the limit. The above studies, though, have made systematic analysis of various impurities in different aspirin brands but in most of these studies, the samples size was low. Therefore, we have made a systematic quantitative analysis of a large number of aspirin samples in terms of active ingredients, toxic heavy metals and salicylic acid impurities.

2. Materials and Methods

2.1. Materials

Salicylic acid (99.8%) was purchased from Riedel-DeHaen, Germany. Acetyl salicylic acid (ASA), analytical grade was obtained from GRR, India. All other reagents used were of analytical grade reagents unless otherwise stated. Heavy

metal standards including: arsenic, cadmium, chromium, lead, were purchased as readymade stock solutions from BDH Spectrosol, UK. Aspirin samples (in the form of tablets) were collected randomly from various pharmacies around Tripoli and Zawia regions. The period of samples collection was scheduled from November 2008 to January 2009. Samples were collected in duplicates containing 300 mg and 100mg active aspirin contents.

2.2. Methods

2.2.1. High Performance Liquid Chromatography (HPLC)

The acetylsalicylic acid (ASA) contents were analyzed on a Shimadzu Model LC-10AS liquid chromatography equipped with SPD-M10A diode array detector and SIL -10A auto injector. An appropriate amount aspirin sample was extracted with a mobile phase solution (Acetonitrile. Methanol and 20 mM sodium phosphate buffer of pH=3, in a portion 50:7:43 v/v). The extracts were made up to the volume (50 ml) with mobile phase and further dilutions were carried out to get a concentration of 30 µg/ml of acetyl salicylic acid. The contents were mixed thoroughly and filtered through 0.45 µm filter paper. An aliquot of 20 µl of both standard and test solutions were injected separately under the described conditions (Figure 1A and B). A stock solution containing 150 µg/ml (150 ppm) of acetylsalicylic acid was prepared by transferring 15 mg of the pure acetylsalicylic acid into 100ml volumetric flask and dissolved in a solution of 50% acetonitrile in water. From this stock solution a series of calibration solutions were prepared containing 20, 40, 60 and 80µg/ml acetylsalicylic acid in the same solvent and the calibration curve was drawn to calculate the concentration of ASA in various aspirin samples (Kirchhoefer & Juhl, 1980, Cham, 1980).

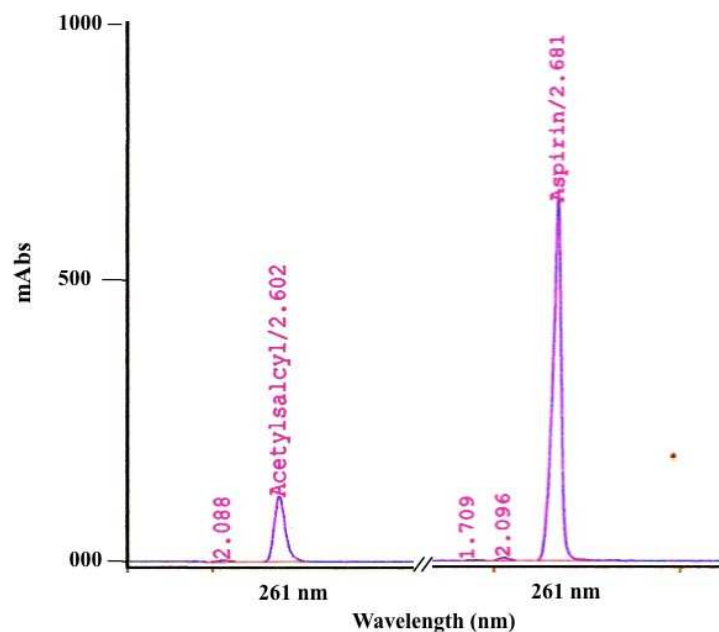


Figure 1. The HPLC response of a standard solution of; A) acetylsalicylic acid. B) HPLC response of an aspirin sample under similar experimental conditions.

2.2.2. Volumetric Titration

The active ASA ingredients in various aspirin samples were also determined using volumetric titration method. A quantity of the powder sample containing 0.5g of aspirin was weighed in a glass beaker and 30ml of 0.5N NaOH was added and the mixture was boiled gently for 10 minutes. The excess of unreacted NaOH was titrated with 0.5N HCl using phenol red solution as indicator. A blank solution containing all the reagents except aspirin was also titrated. The difference between the titrations represents the amount of NaOH consumed by the aspirin (acetylsalicylic acid). Therefore, each ml of NaOH consumed is equivalent to 0.04504 gram of $C_9H_8O_4$. The procedure was repeated three times for each sample and the results were analyzed by taking the average.

2.2.3. Atomic Absorption Spectroscopy

Toxic heavy metal impurities were analyzed using atomic absorption spectroscopy performed on a Shimadzu type AA6701F atomic spectrophotometer attached to a graphite furnace and out-injector (ASC6000). This method has been used to determine heavy metal contents in various chemical preparations. All the experiments were run in triplicate and the data were collected using automatic processor attached to a PC.

Working solutions of trace elements were prepared daily by appropriate dilution (0, 1, 5, 10, 15 and 20 ml) of standard heavy metal stock solution (10 ppm). The solutions were transferred to each of six 100 ml volumetric flasks and 5 ml of concentrated HNO_3 was added to each flask. The volumes were completed with deionized water to 100 ml giving 0, 0.1, 0.5, 1.0, 1.5 and 2ppm concentration of each heavy metal. A calibration curve was drawn by running serial dilutions of the standard heavy metals as described by manufacturer. The corresponding absorption values were recorded and fitted by using linear regression method (Figure 1). The concentration of trace elements in various aspirin samples was calculated from the calibration curve using the 'slope' and 'intercept' values obtained by linear regression method. Aspirin samples were prepared by dissolving an appropriate powder of aspirin tablets in 10ml of concentrated nitric acid. The mixture was then carefully heated on hot plate to dryness. Then 5 ml of hydrogen peroxide was added and heated again to dryness. The residue was then treated with 20 ml of 0.1 N HNO_3 and stirred for 10 minutes. The sample solution was then filtered into 50 ml volumetric flask and the volume was made with double distilled water.

2.2.4. Fluorometry

The fluorimetric analysis for salicylic acid in aspirin samples was carried on a Shimadzu Model Rf-5301 PC spectrofluorometer. The instrumental conditions used were set as follows: Excitation wavelength λ_{Ex} =298.0nm and Emission wavelength λ_{Em} =410.0nm. The calibration curve was constructed using the pre described standards using (1cm) quartz cell. The aspirin samples were then measured for the salicylic acid impurities as follow. A 20mg sample of the fine

aspirin powder was transferred into 250ml volumetric flask. 40ml of sodium fluoride-hydrochloric acid solution was added and the mixture shaken for five minutes. After 10 minutes with frequent shaking the solution mixture was diluted to the volume using buffer solution pH=4. The solution was then filtered through whatman filter paper (No: 541) and 2ml of the filtrate was diluted to 100 ml with the buffer and this solution was measured fluorometrically at 410 nm (White & Weissler, 1972).

A stock solution containing 10 μ g/ml (150 ppm) of salicylic acid was prepared by transferring 10mg of the pure salicylic acid into a 250ml volumetric flask and dissolved in 40ml of sodium fluoride-hydrochloric acid solution after frequent shaking for 15 minutes the volume was completed to the mark using buffer pH=4. From this stock solution a series of calibration solutions were prepared containing 0, 2, 5, 20 and 60 μ g/ml salicylic acid in the same solvent.

3. Results and Discussion

3.1. Acetylsalicylic Acid Contents

The acetylsalicylic acid contents (ASA) were determined using HPLC and volumetric titration. First, linearity and purity of the standard ASA solutions was established on HPLC column. Fig. 1A shows the HPLC elution profile of a standard solution of ASA. As can be seen from the Fig. 1A that the response of standard ASA on HPLC column was linear and gave a single peak within the expected range. Then triplicate injections of several standard solutions of ASA were loaded on to the HPLC column and the mean peak areas were taken into account to draw the calibration curve. Various aspirin samples were then eluted through the same HPLC column and each sample gave a single peak within the expected range as show in Fig. 1B. The peak area of all the aspirin samples was then measured, and using the calibration curved drawn from the above standards, contents of ASA in various aspirin samples were calculated and the results are shown in the table 1 (middle column). The results of ASA contents as obtained by HPLC were further confirmed by using volumetric titration method and the data is shown in table 1 (right column). As can be seen from the table 1, that the ASA contents in various aspirin samples obtained by titration method were more or less similar to that obtained by HPLC methods. Further, as can be seen from the table 1 that the range ASA contents in various aspirin samples varies from 87.70 - 104%. Except, two samples (AS6, AS7), which contained <90% ASA contents, remaining aspirin samples were in good agreement and and contained >90% active ASA ingredients. Further, shown in table 2 are the comparison of the ASA contents formulated by the respective pharmaceutical company and the actual ASA contents determined in the laboratory. As can be seen from the table 2 that ASA contents of most of the samples formulated by the companies showed deviation from those determined in the laboratory, but were within the permissible limits. These results are in agreement with various reports on the active ASA

contents in commercial aspirin preparation (Franeta et al, 2002, Salako et al., 1989, Juhl & Kirchhoefer, 1980, Cham, 1980).

Table 1. Table shows the comparison of the acetylsalicylic acid content in various aspirin samples determined by both volumetric titration method and HPLC.

Sample no.	Acetylsalicylic acid (%)	
	Determined by HPLC method	Determined by titration method
AS1	98.4±1.33	97.49±0.76
AS2	87.9±3.21	100±1.07
AS3	97.0±1.21	97.0±1.91
AS4	99.8±1.0	100±1.6
AS5	99.4±0.87	99.8±0.87
AS6	87.8±3.41	93.6±4.81
AS7	87.7±2.22	104 ±2.09
AS8	97.4±0.81	105±3.32
AS9	92.0±4.62	92.0±3.42
AS10	96.2±2.06	96±3.08
AS11	93.2±4.55	93.2±4.02
AS12	101±0.78	101±2.08
AS13	99.0±1.66	99.0±1.23
AS14	95.0±0.21	95.0±0.01

Table 2. Comparison of acetylsalicylic acid (ASA) contents formulated by the pharmaceutical companies with the actual ASA contents determined in the laboratory.

Sample no.	Formulated in the company	Determined in the laboratory
AS1	300mg	292.5mg
AS2	300mg	303.0mg
AS3	300mg	291.0mg
AS4	300mg	300.0mg
AS5	300mg	299.4mg
AS6	300mg	288.9mg
AS7	300mg	312.0mg
AS8	320mg	336.0mg
AS9	300mg	276.0mg
AS10	100mg	96.3mg
AS11	75mg	69.9mg
AS12	100mg	101.0mg
AS13	81mg	80.20mg
AS14	81mg	76.9mg

3.2. Toxic Heavy Metal Impurities

We next examined the toxic heavy metal impurities in various aspirin samples. The four heavy metal of serious health concern, which includes: lead, chromium, cadmium, and arsenic were analyzed using graphite furnace atomic absorption spectroscopy. The analysis of the samples was made in triplicate and the average value was used in calculation. Fig. 2, shows the calibration curve of standard heavy metal drawn by using atomic absorption spectroscopy. Using this calibration curve, concentration of four toxic heavy metals namely; lead, cadmium, arsenic, and chromium in various aspirin brands were determined and the results are shown in the table 3. The results indicate that all the determined heavy metals were within the permissible limit directed by the world health organization (WHO; The International Pharmacopeia, 2011). However, variation between the concentrations of the heavy metals in all the samples was obvious. The level of lead was the highest

among the rest and ranged from 0.002 to 0.054ppm. Chromium ranked the second in its concentration levels and ranged from <0.001 to 0.028ppm. As can also be seen from the table 3 that two samples (AS9 and AS12) contain the highest concentrations of lead and chromium (table 3). Comparing our obtained results with those previously obtained by the work of Miyoshi et al., (2009), they found the concentration of chromium ranged from <24 to 658µg/kg in aspirin samples, where as in our results chromium concentration ranged from <1.0 to 28 µg/kg. Nevertheless, in both cases the concentration of chromium and other heavy metals was with the permissible limits.

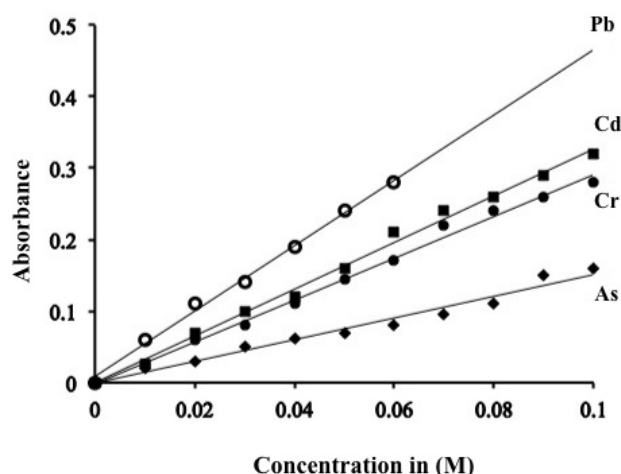


Figure 2. Calibration curves for the standard heavy metals; lead (Pb), cadmium (Cd), chromium (Cr) and arsenic (As). The value of 'slope' and 'intercept' were calculated by linear regression method and used to determine the concentration of heavy metals in various aspirin brands.

Table 3. Table shows the concentrations of toxic heavy metals impurities in various commercial aspirin preparation determined by atomic absorption spectroscopy.

Sample no.	Heavy metal concentration (µg/g)			
	Pb	Cr	Cd	As
AS1	0.010	0.011	0.001	0.001
AS2	0.003	<0.001	0.005	<0.001
AS3	0.017	<0.001	0.010	0.003
AS4	0.003	<0.001	<0.001	<0.001
AS5	0.003	<0.001	<0.001	<0.001
AS6	0.006	<0.001	0.012	<0.001
AS7	0.002	<0.002	0.016	<0.001
AS8	0.017	0.009	<0.001	0.001
AS9	0.054	0.015	<0.001	0.003
AS10	0.016	0.016	<0.001	0.001
AS11	0.015	0.017	<0.001	0.005
AS12	0.008	0.028	<0.001	<0.001
AS13	0.013	0.019	<0.001	0.002
AS14	0.002	0.018	<0.001	<0.001

3.3. Salicylic Acid Impurities

The second possible impurity determined in various aspirin tablets is the salicylic acid percentage. Table 4, shows the fluorometric analysis of salicylic acid percentage in the aspirin samples, which ranged from 0.0906% to 0.138%. The results indicate that the salicylic acid impurities were within the permitted levels. The upper limit is 0.3% as suggested by

Bamigbola et al. (2009). Juhl and Kirchhoefer (1980) found that 10% of the studied samples failed to meet the permitted limit of salicylic acid impurities in their study. Salako, et al. (1989) also found that some of the samples exceeded the limit as they analyzed twelve aspirin samples.

In conclusion, the results presented in this study suggest that active ASA ingredients vary for most of the collected aspirin samples, however; they were not of great significance. Nevertheless, they trigger the need for quality control assurance for all types of medicine. Furthermore, active ASA ingredients as determined by HPLC and volumetric titration method showed significant variations among samples but in all the cases ASA contents in aspirin samples were within the permissible limits. Trace element impurities in various aspirin samples were also found within the permissible limits as set by world health organization (WHO, 2011), however; variations exist among various aspirin samples. Similarly, salicylic acid impurities observed in most of the aspirin samples were also found within the permitted limits, however; variation exists among various aspirin samples.

Table 4. Table presents the percentage of the salicylic acid impurities in the aspirin samples determined by fluorometric spectroscopy.

Sample no.	Salicylic acid (%)
AS1	0.0906
AS2	0.0920
AS3	0.1116
AS4	0.1073
AS5	0.1381
AS6	0.1049
AS7	0.0965
AS8	0.1033
AS9	0.1228
AS10	0.1289
AS11	0.0951
AS12	0.1061
AS13	0.1123
AS14	0.1160

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