Evaluation of the Spectroscopic Methods for the Determination of Acetyl Salicylic Acid Concentration

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A Dissertation
Submitted to the University of Gezira in Partial Fulfillment of the for the Requirements Degree of Master of Science in Chemistry

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First and foremost thanks to **Allah Almighty** for his uncountable grace, blessings and enlightenment.

I would like to acknowledgment with thanks and gratitude my supervisor **Dr. Fath Elrahman Abbas Elsheikh** for his help and patience. I would like to special thanks to the **Dr. Amad Osman Aboraid** for his invaluable and enormous amount of patience, assistance and guidance during this research.

I would like to thank the laboratories and staff of Faculty of Pharmacology department – University of Medical Sciences and Technology.
Dedication

To my dearest mother ......................

To my brothers and my sister ..............

To all my family ............................

I dedicate this humble work .................
Evaluation of the Spectroscopic Methods for the Determination of Acetyl Salicylic Acid Concentration
Amal Ali Mohammed Ali
Master of Science in Applied Chemistry Department of Applied Chemistry and Chemical Technology
Faculty of Engineering and Technology
University of Gezira

Abstract

Acetylsalicylic acid one of the important compounds that is used in pharmaceutical industry to relief pain and fever. The purpose of this study aimed to measure how humidity affects the stability of Acetyl Salicylic Acid. Acetyl Salicylic Acid dissociates by means of humidity to salicylic acid and acetic acid. To measure the degradation of Acetyl Salicylic Acid we used the Spectrophotometer instrument to determine the absorbance two equi-concentration solution of Acetyl Salicylic Acid was prepared. One of the samples was treated by sodium hydroxide and other sample was left untreated. The treated sample was boiled in a water bath for ten minutes then cooled. Then two solution reacted with Fe III, since Fe III reacts with salicylic acid only the colour formed with the untreated solution will represent the mount of salicylic acid present in the sample as degradation while the colour formed with the hydrolyzed solution is the total salicylic acid in the a Acetyl Salicylic Acid. The absorbance of each treated and an treated solution was measured at the wavelength of maximum absorption at 530 nm against its corresponding untreated solution. Then the results show that linearity was calculated and it was found that the intercept 0.0263, Slope 9.0575 correlation coefficient 0.9997. Precision was also evaluated and the results were found to be Mean 99.64, Standard Deviation 0.95, and Relative standard Deviation 0.951. Repeatability Results day (1&2) showed that the Mean 100.89 Standard Deviation was 1.14, and the Relative Standard Deviation was 1.135 Recovery results were as follows: Average 101.03, Standard Deviation 0.44, Relative Standard Deviation, Range from 0.0146 to 0.0149 and intercept from 0.3730 to 0.4210. Specificity the results of spiking Acetyl Salicylic Acid with varying amounts of salicylic acid ranging from 12.5% to 62.5%. Thus it is concluded that the proposed method of analysis is new, simple, cost effective, environment friendly, accurate rapid and reproducible and this method can be successfully employed in the routine analysis of Acetyl Salicylic Acid in tablet formulation. It is recommended that this method may be used as standard one.
استيل سالسليك اسد من المركبات الهامة شائعة الاستعمال لأزالة الالام والحمى . الاسم العلمى 
استيل سالسيلك اسيد ، هدفت هذه الدراسة قياس مدى تأثير الرطوبة على ثبات استيل سالسليك اسد الذي 
يتحلل بفعل الرطوبة إلى سالسليك اسيد واسيد. لقياس تحلل استيل سالسليك اسد نستعمل جهاز 
التحليل الضوئي تم تحضير عينتان من محلول استيل سالسليك اسد العينة الأولى معالجة بواسطة 
هاييدروكسيد الصوديوم أما العينة الثانية غير معالجة بواسطة هاييدروكسيد الصوديوم العينة المعالجة تسخن 
في حمام مائي لمدة 10 دقائق ثم تبرد. نضيف الحديد 
إلى العينتين المعالجة وغير المعالجة بما أن 
الحديد يتفاعل مع حمض السالسيلك الناتج من تحلل استيل سالسليك اسد بفعل الرطوبة ولا يتفاعل مع حمض السالسليك المرتبط مع استيل سالسليك اسد أذن اللون البنفسجي الذي يظهر في العينة غير 
المعالجة يمثل كمية حمض السالسليك الناتجة عن التحلي بفعل الرطوبة بينما اللون البنفسجي الذي 
يظهر في العينة المعالجة يمثل كمية حمض السالسليك الكلية الموجود في استيل سالسليك اسد الناتجة من 
تحلل استيل سالسليك اسد بفعل الرطوبة والمرتبطة مع استيل سالسليك اسد. ادخلت العينتان في جهاز 
التحليل الضوئي وتم قياس الامتصاص عند في الطول الموجي 530 نانوميتر. أذن الفرق في الامتصاصية 
بين المحلولين في أقصى طول الموج ممثلاً كمية حمض سالسليك المرتبطة مع استيل سالسليك اسد
ووجد في التجربة الخلوية أن التقلات 0.0263 والميل 9.575. وفي اختبار الدقة وجد الوسط الحسابي 99.64 
والانحراف المعياري 0.95 والاكتاف 0.951 واستفاد الوصول الوسط الحسابي 101.03 
والانحراف المعياري 0.44 والاكتاف 0.0146 إلى 0.0149 والانحراف بين 0.3730 إلى 0.4210. ونجد في التجربة الاقتصادية تحديد تحلل استيل سالسليك اسد في وجود حمض السالسليك اسد وذلك باضافة كمية مختلفة من السالسليك اسيد إلى استيل سالسليك اسد ووجد أن المعدل بين 12.5% إلى 62.5% من خلال هذه التجارب اثبتت أن طريقة التحليل بواسطة جهاز التحليل الضوئي 
بأنها دقيقة وسريعة و-------------------- وبسيطة وغير مكلفة وصديقة للبيئة ويمكن استخدامها بنجاح في تحليل حبوب
الاسبرين، ومن خلال قياس مدى تأثير الرطوبة على ثبات الاسبرين الذي يتحلل بفعل الرطوبة إلى سالسيلك اسيد واستيكل اسد نوصي بأن هذه الطريقة قياسية.
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Chapter One

Introduction and Literature Review
1. Introduction and Literature Review

1.1 History of Acetyl Salicylic Acid

Acetyl salicylic acid, (ASA), was first synthesized in 1893 by Felix Hofmann, a chemist worked in the German firm of Bayer. This compound had the medicinal properties of salicylic acid, an extract of willow bark, without the unpleasant taste or the high degree of irritation of the mucous membranes lining the mouth, gullet and stomach (1).

1.2 Synthesis of Acetyl Salicylic Acid

The synthesis of Acetyl salicylic acid is an esterification reaction. Salicylic acid is treated with acetic anhydride, an acid derivative, causing a chemical reaction that turns salicylic acid's hydroxyl group into an acetoxy, (R-OH → R-OCOCH3). This process yields Acetyl salicylic acid and acetic acid, which is considered a byproduct of this reaction as shown in Figure (1). Small amounts of sulfuric acid (and occasionally phosphoric acid) are almost always used as a catalyst (2).

![Figure (1) Synthesis of Acetyl salicylic acid](image-url)
1.3 Physical properties of Acetyl Salicylic Acid

Acetyl salicylic acid is a white, crystalline, weakly acidic substance, with a melting point of 135 °C (275 °F). Acetylsalicylic acid decomposes rapidly in solutions of ammonium acetate or of the acetates, carbonates, citrates or hydroxides of the alkali metals. Acetylsalicylic acid is stable in dry air, but gradually hydrolyses in contact with moisture to acetic and salicylic acids. In solution with alkales, the hydrolysis proceeds rapidly and the clear solutions formed may consist entirely of acetate and salicylate (3). Formulations containing high concentrations of Acetyl salicylic acid often smell like vinegar due to the degradation product acetic acid (4).

The acid dissociation constant (pKa) for acetylsalicylic acid is 3.5 at 25 °C (77 F) (5).

1.4 Stability of Acetyl Salicylic Acid

Acetyl salicylic acid is stable in dry air, but readily hydrolyzes to acetate and salicylate when exposed to water or moist air (Figure 2). It will then exude a strong vinegar-like odor. The addition of heat will speed the rate of hydrolysis. In aqueous solutions, Acetyl salicylic acid is most stable at pH values of 2-3 and least stable at pH values below 2 or greater than 8 (10).

Figure (2) Hydrolysis of Acetyl Salicylic Acid
An early study of pH-profile was applied to Acetyl salicylic acid overall velocity constant for Acetyl salicylic acid hydrolysis at 17°C as a function of pH (17). Hydrolysis of Acetyl salicylic acid to salicylic acid was of concern to the pharmacologist Dressor, who in 1899 prepared what nowadays one would call a preformulation profile. He tested the hydrolysis of Acetyl salicylic acid in acid and alkaline solutions and determined hydrolysis constants of $3.3 \times 10^{-4}$ at body temperature in acid medium and $2.5 \times 10^{-2}$ at room temperature in alkaline medium. He also observed that Acetyl salicylic acid was easily split in weakly alkaline medium which had consequences for the metabolism. He established the first profile for the pH dependence of hydrolysis of Acetyl salicylic acid. Of course, the definition of pH was unknown at the time (10).

The test for free salicylic acid is a required test on all samples of Acetyl salicylic acid powder. Acetyl salicylic acid hydrolysis takes place even when the drug is in the solid powder form. The hydrolysis of Acetyl salicylic acid involves the rupture of a covalent bond between a carbon atom and oxygen atom.

This happens in the presence of water and much faster when either an acid or an alkali is present. Acids, alkalies and certain enzymes, which are capable of supplying the hydrogen or hydroxyl ions to the reaction mixture catalyse this hydrolysis. The alkaline hydrolysis of an ester is irreversible while an acid hydrolysis is reversible. The Acetyl salicylic acid hydrolysis rate increases in direct proportion to the water vapor pressure in an environment. Coating process subjects ASA tablets to both high temperatures and humidity and thus to more degradation so it is important that the formulation is resistant to moisture interaction (18).

Many studies concerning Acetyl salicylic acid stability were performed. During manufacturing certain hydrophilic disintegrants combine with ASA under high humidity conditions (19).
Further work was applied to study aqueous coating solutions effect on ASA tablets. The results confirmed that moisture penetration during the coating process was not only formulation dependent but could be directly linked to the stability of the final coated ASA tablet (18). The effect of alkaline moieties of the antacid on the chemical stability of Acetyl salicylic acid was studied. The results revealed that the presence of the alkaline moieties in the tested tablets has increased the rate of Acetyl salicylic acid decomposition and reduces its shelf-life (20).

The percentage of hydrolysis is higher in effervescent and buffered tablets, as a result the limit of salicylic acid listed in the pharmacopoeia is higher than compressed Acetyl salicylic acid tablets (10). Effervescent tablets are uncoated tablets that generally contain acid substances and carbonates or bicarbonates, and that react rapidly in the presence of water by releasing carbon dioxide. Effervescent products are not stable in the presence of moisture. Most effervescent products are hygroscopic and can therefore adsorb enough moisture to initiate degradation if they are not suitably packaged (21).

Acetyl salicylic acid tablets should be stored in tight, moisture resistant containers with suitable conditions of temperature and moisture. Products should not be used if a strong vinegar-like odor is noted emitting from the bottle of Acetyl salicylic acid. (22,23).

1.5 Assay of Acetyl Salicylic Acid

The analysis of Acetyl salicylic acid with its precursor and degradation product salicylic acid, is difficult. Residual salicylic acid was determined by the convenient reaction with ferric salts (typical for phenols), which gives a violet complex with salicylic acid. Ferric salts are described for identification of Acetyl salicylic acid after being hydrolyzed (22,23).
In USP Acetyl salicylic acid as a bulk drug is assayed by titration with 0.5N sulphuric acid after pretreatment with 0.5 N NaOH, using phenolphthalein as indicator. Acetyl salicylic acid tablets are assayed by liquid chromatography using mobile phase which is prepared by dissolving 2g of sodium 1-heptansulfonate in a mixture of 850 ml of water and 150 ml of acetonitrile and adjusting pH with glacial acetic acid to 3.4. The solvent system is a mixture of acetonitrile and formic acid (99:1), the liquid chromatograph is equipped with a 280-nm detector and a 4.0-mm x 30-cm column containing packing L1 (23). Acetyl salicylic acid as a bulk drug and tablets are assayed in the BP by titration with 0.5M hydrochloric acid after pre-treatment with 0.5 M sodium hydroxide using phenolphthalein solution as indicator and ethanol used as a solvent (22).

A rapid, simple assay procedure was developed for simultaneous analysis of ASA and SA in Acetyl salicylic acid delayed-release tablet formulation by zero crossing second-derivative UV spectrophotometry. The zero-order absorption spectra and second derivative spectra of ASA and SA were recorded in diluted solution acetonitrile : formic acid (99:1). The accuracy of the method was demonstrated by the determination of ASA and SA in five tablets formulations (each 20 tablets of the same batch) by the described method and by high performance liquid chromatographic method. The results were in good agreement(24).

A recent, simple, accurate, and precise HPTLC method has been established for separation and simultaneous analysis of Acetyl salicylic acid, salicylic acid, and sulfosalicylic acid. standard and sample solutions of Acetyl salicylic acid, salicylic acid and sulfosalicylic acid were applied to aluminum foil silica gel G 60 F254 HPTLC plates impregnated with 2% ( w/v ) boric acid in ethanol. The plates were developed with chloroform-methanol-ammonia-water 120:75:2:6 (v/v) as mobile phase. UV detection was performed at 254 nm (25).
An effective separation of paracetamol, caffeine and Acetyl salicylic acid was achieved by using adsorbents with immobilized nitrile groups. The effects of the concentration of acetonitrile and potassium phosphate in the mobile phase and pH of the mobile phase on the retention time of analytes and a possible additive like salicylic acid has also been studied. In comparison to a column with a C18 adsorbent used earlier for routine analysis, the chromatograms obtained were characterized by higher separation efficiency, while the proposed separation procedure is more cost-effective and rapid (26).

Simultaneous spectrophotometric analysis of a ternary mixture containing Acetyl salicylic acid, paracetamol and caffeine was achieved. The absorbance of a solution of the mixture in 0.01 M hydrochloric acid was recorded at wavelengths 229, 244 and 274 nm. (26).

1.6 Impurities of Acetyl Salicylic Acid

Hydrolysis of Acetyl salicylic acid can be a major factor in the stability of the drug. Acetyl salicylic acid undergoes hydrolysis with the resultant degradation products being salicylic acid and acetic acid(10).

1.6.1 Salicylic acid

Salicylic acid occurs as white, fine needle-like crystals or as a fluffy, white, crystalline powder. It is stable in the air (23). Not only salicylic acid but all the salicylates cause severe toxicity (salicylism). The most toxic salicylate is oil of wintergreen (methyl salicylate). One teaspoon of 98% methyl salicylate contains 7000 mg of salicylate, the equivalent of nearly 90 baby Acetyl salicylic acid tablet and more than 4 times the potentially toxic dose for a child who weighs 10 kg. The early symptoms of salicylism are CNS stimulation with vomiting, hyperpnoea, hyperactivity, hyperthermia and even convulsions. This quickly turns to depression, with lethargy, respiratory failure and collapse (10).
1.6.2 Assay of salicylic acid

Salicylic acid limits according to USP are (NMT 0.1%) in bulk drug, (NMT 0.3%) in compressed Acetyl salicylic acid tablets, (NMT 3.0%) in effervescent and buffered Acetyl salicylic acid tablets and (NMT 4.0%) in coated tablets. Salicylic acid is assayed in USP by liquid chromatography using the same mobile phase and solvent system that previously mentioned in the assay of Acetyl salicylic acid tablets (23).

The BP describes limits for salicylic acid (NMT 0.1%) in bulk drug, (NMT 3.0%) in Acetyl salicylic acid tablets of different dosage forms. Salicylic acid is assayed in BP by color test using ammonium ferric sulphate solution that resulted in the formation of a complex with a violet colour (23).

Spectrophotometric determination of salicylic acid in Acetyl salicylic acid was described by Wahbi et al. A portion of powdered tablets or bulk Acetyl salicylic acid was extracted with ethanolic 1% monochloracetic acid. After filtration, the first derivative spectrum of the solution, was recorded. The trough at 316 nm was used for the determination of salicylic acid (27).

Determination of salicylates in biological fluids is of interest for several purposes. It can be done by several methods like gas-liquid chromatography (GLC). High performance liquid chromatography (HPLC) is an alternative to GLC but more complex and time consuming.

Spectrophotometry is the earliest and most widely used method for measuring serum salicylate level. Colorimetry is a method where salicylic acid is determined by measuring the absorbance of the ferric ion salicylate complex, it is simple, inexpensive, rapid and very reliable, however it measures salicylates rather than ASA (28,29).
1.7 Principle of the method

The most commonly used method for the determination of acetylsalicylic acid involves its quantitative hydrolysis in a basic medium to yield the salicylate dianion, equation (1), acidification to the monoanion, equation (2), and finally complexation with Fe(III) to yield the Purple colored tetraaquosalicylatoiron(III) complex, equation (3). Although very commonly used, this method lacks the specificity and accuracy required especially when it is to be used in post-marketing surveillance studies or during the stability testing of Acetyl salicylic acid tablets since it does not distinguish between the salicylic acid already present in the sample as impurity/degradation product and that produced by hydrolysis of Acetyl salicylic acid.

In the proposed method two equi-concentration solutions of Acetyl salicylic acid, prepared from the same material will be reacted with Fe (III), one of the solutions will contain Acetyl salicylic acid hydrolyzed using sodium hydroxide solution and the other solution is untreated Acetyl salicylic acid solution. Since Fe (III) reacts with salicylic acid only and not the intact Acetyl salicylic acid, the colour formed with the untreated solution will represent the amount of salicylic acid present in the sample as impurity/degradation while the colour formed with the hydrolyzed solution is the total of both intact Acetyl salicylic acid originally present and salicylic acid in the form of degradation/impurity product, therefore the absorbance difference of the two solutions at the wavelength maximum absorption of the colour complex will represent the amount of intact Acetyl salicylic acid only.
Figure (3) The reaction scheme for the determination of Acetyl Salicylic Acid
1. General Objectives

To develop stability indicating assay method for the determination of Acetyl salicylic acid only in presence of its degradation product (salicylic acid).

To validate the developed method with regard to its linearity, accuracy (recovery), specificity and precision.

To use the developed method for the analysis of Acetyl salicylic acid only tablets formulation
2. Materials and Methods

2.1 Materials

2.1.1 Chemicals and reagents

- Acetyl salicylic acid only (acetylsalicylic acid) and Salicylic acid (were gift from Amipharma, Sudan).
- Acetyl salicylic acid only tablets (From, comercial).
- Ferric chloride hexahydrate (Lab Teach Chemical - India).
- Sodium hydroxide pellets (BDH Laboratory Supplies - England).
- Ethanol absolute (SD Fine Chemicals - India).
- Sodium hydroxide in aqueous solution: Prepared by dissolving 40 gm of sodium hydroxide pellets in 1000 ml of distilled water.
- Ferric chloride solution: Concentration 5.44 mg/ml 5.44 g ferric chloride hexaydrate and were transferred quantitatively to a 1 liter volumetric flask, about 100 ml distilled water were added to dissolve the solid followed by 3 ml concentrated hydrochloric acid. The volume of the solution was then made to mark using distilled water.

2.1.2 Equipment

- Analytical balance (Voyager, oHAus Switzerland)
- Thermostated water bath (Gesellschaft Fur la Ph METER (Ph 523, made in west Germany)
- UV/Visible spectrophotometer (Modeluv 1800 ENG 240 V, SOF shimadzu corporation, Japan)
2.2 Spectrophotometric Methods of Analysis for Drugs in Combination

Simultaneous estimation of drug combination is generally done by separation using chromatographic methods like HPLC, GC and HPTLC etc. These methods are accurate and precise with good reproducibility, but the cost of analysis is quite high owing to expensive instrumentation, reagent and expertise. Hence it is worthwhile to develop simpler and cost effective method for simultaneous estimation of drugs for routine analysis of formulation. Spectrophotometric analysis fulfils such requirement where the simultaneous estimation of the drug combination can be done with similar effectiveness as that of chromatographic methods.

The spectrophotometric assay of drugs rarely involves the measurement of absorbance of samples containing only one absorbing component. The pharmaceutical analyst frequently encounters the situation where the concentration of one or more substances is required in samples known to contain other absorbing substances, which potentially interfere in the assay. If the formula of the samples is known, the identity and concentration of the interfering substance are known and the extent of interference in the assay may be determined.

A number of modifications to the simple spectrophotometric procedure are available to the analyst, which may eliminate certain sources of interference and permit the accurate determination of all of the absorbing components. Each modification of the basic procedure may be applied if certain criteria are satisfied.

The basis of all the spectrophotometric techniques for multicomponent samples is the property that at all wavelengths:

- the absorbance of a solution is the sum of absorbance of the individual components or
- the measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference cell. There are various spectrophotometric methods are available which can be used for the analysis of a combination samples. Following methods can be used
  - Simultaneous equation method
  - Derivative spectrophotometric method
  - Absorbance ratio method (Q-Absorbance method)
  - Difference spectrophotometry
  - Solvent extraction method

2.3 Experimental
2.3.1 Linearity
2.3.1.1 Stock solutions preparation
  Accurately weighed about 400mg of Acetyl salicylic acid only were dissolved it in 50 ml of ethanol. 10 ml aliquots from this solution were transferred to each of two 100 ml volumetric flasks. To one of the flasks 10 ml 0.1M NaOH were added, while the other flask was left untreated, the treated standard solution was boiled in a water bath for 10 minutes, cooled and each of the two flasks was made to volume with distilled water.

2.3.1.2 Calibration series
  0.012, 0.024, 0.036, 0.048 and 0.060 mg / ml aliquots from each of the two stock solutions (the treated and untreated solution) were transferred to a set of 50 ml volumetric flasks, the volume of each flask was made to mark with 0.02 M ferric chloride solution.

2.3.1.3 Absorbance measurement
  The absorbance of each treated solution was measured at wavelength of maximum absorption at 530 nm against it is corresponding untreated solution in the blank cell.
The measured absorbance readings were plotted against their corresponding concentrations; the slope and the intercept of the plotted lines were obtained by least squared method using Microsoft Excel Spreadsheet. The plot of the versus concentrations their corresponding residuals was also made.

2.3.2 Precision

2.3.2.1 Repeatability

Twenty tablets were weighed and the average weight per tablet was determined. The tablets were then finely ground. From the powder mass, weights equivalent to one tablet were taken, each was transferred to a 50 ml volumetric flask, dissolved using ethanol and filtered through Whatman No. 41 paper. From the filtrate of each sample 10 ml volume was transferred to each of two 100 ml volumetric flasks. The two samples set was handled in a similar manner as mentioned under 2.3.1.1 (Stock solutions preparation).

5ml of each of the treated and the untreated sample were transferred to each of two 50 ml volumetric flask, the volume in each flask was made to mark with 0.02 M ferric chloride solution. The absorbance of each treated sample set was measured at wavelength of maximum absorption 530 nm against its corresponding untreated solution placed in the blank cell.

The content of Acetyl salicylic acid only per tablet was determined using Acetyl salicylic acid only standard solution prepared using 5 ml from the stock solution used for linearity study, treated in the same manner as mentioned in 2.3.1.2.
2.3.2.2 Intermediate precision

The same procedure was repeated in a different day using freshly prepared reagent made on the same day, to estimate between–day precision.

2.3.3 Recovery

Since the tablets matrix is not known, the method of standard addition was used to study the method recovery. This experiment was repeated three times using different sample weight every time.

2.3.3.1 Spiking standard solution

Accurately weighed about 400mg of Acetyl salicylic acid only were dissolved it in 50 ml of ethanol. 10 ml aliquots from this solution were transferred to each of two 100 ml volumetric flask. To one of the flasks 10 ml 0.1M NaOH were added, while the other flask was left untreated, the treated standard solution was boiled in a water bath for 10 minutes, cooled and each of the two flasks was made to volume with distilled water.

2.3.3.2 Sample stock solutions

Twenty tablets were weighed and the average weight per tablet was determined. The tablets were then finely ground, from the powder mass weights equivalent to one tablet were taken, each was transferred to a 50 ml volumetric flask, dissolved using ethanol and filtered through Whatman No. 41 paper. From the filtrate 5 ml volume was transferred to each of two 100 ml volumetric flasks. The two samples set was handled in a similar manner as mentioned under 2.3.3.1.
2.3.3.3 Spiked samples preparation

- Equal volumes of the treated sample stock solution were spiked with different volumes of the treated spiking standard solution according to the following scheme:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Sample volume (ml)</th>
<th>Spiking std volume (ml)</th>
<th>Final volume (ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>5</td>
<td>50</td>
</tr>
</tbody>
</table>

- made with 0.02 M ferric chloride solution
- untreated spiked samples solution were prepared using the same approach followed in the previous step, replacing the treated sample stock solution and the treated spiking standard solution with the untreated ones.

The absorbance of each treated sample was measured at wavelength of maximum absorption 530 nm against it is corresponding untreated one placed in the blank cell.

The obtained absorbance readings of samples were plotted versus the concentrations of the spiking standard used.
2.3.4 Specificity

2.3.4.1 Acetyl Salicylic Acid stock solution

Accurately weighed 400mg of Acetyl salicylic acid were dissolved it in 50 ml of ethanol. 10 ml aliquots from this solution were transferred to each of two 100 ml volumetric flask. To one of the flasks 10 ml 0.1M NaOH were added, while the other flask was left untreated, the treated standard solution was boiled in a water bath for 10 minutes, cooled and each of the two flasks was made to volume with distilled water.

2.3.4.1 Salicylic acid stock solution

Accurately weighed about 40 mg salicylic acid were dissolved in 100 ml of ethanol.

To three sets of 50 ml volumetric flasks containing equal volumes of Acetyl salicylic acid stock, solution different volumes from the salicylic acid stock solution were added according to the following scheme:

<table>
<thead>
<tr>
<th>Salicylic acid std</th>
<th>Treated Acetyl Salicylic Acid std</th>
<th>Untreated Acetyl Salicylic Acid std</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml</td>
<td>4 ml</td>
<td>4 ml</td>
</tr>
<tr>
<td>2 ml</td>
<td>4 ml</td>
<td>4 ml</td>
</tr>
<tr>
<td>5 ml</td>
<td>4 ml</td>
<td>4 ml</td>
</tr>
</tbody>
</table>
The volume of each flask was completed to mark with 0.02 M ferric chloride solution

The absorbance of each treated sample was measured at wavelength of maximum absorption 530 nm against its corresponding untreated one, placed in the blank cell

The percentage content of Acetyl salicylic acid in each sample was determined using 4 ml from the Acetyl salicylic acid stock standard treated in the same manner as shown in the table, but ignoring the addition of salicylic acid standard.
3. Results and Discussion

3.1 Linearity

The plot of Acetyl salicylic acid Absorbance against their corresponding concentration gave straight line with equation \( y = 9.575 \times + 0.0263 \) as shown in Figure 5, the regression parameters of the straight line equation are shown in Table 1.

The plot of the concentrations versus their corresponding residuals is not showing any trend. This further supports the linear relation between the concentration and the absorbance values obtained as shown in Figure 6.

<table>
<thead>
<tr>
<th>Concentration mg/ml</th>
<th>Absorbance Calculated</th>
<th>of Absorbance</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.012</td>
<td>0.145</td>
<td>0.1412</td>
<td>0.0038</td>
</tr>
<tr>
<td>0.024</td>
<td>0.256</td>
<td>0.2561</td>
<td>0.0001</td>
</tr>
<tr>
<td>0.036</td>
<td>0.364</td>
<td>0.371</td>
<td>-0.007</td>
</tr>
<tr>
<td>0.048</td>
<td>0.485</td>
<td>0.4859</td>
<td>-0.0009</td>
</tr>
<tr>
<td>0.060</td>
<td>0.605</td>
<td>0.6008</td>
<td>0.0042</td>
</tr>
</tbody>
</table>

**Regression Parameters**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Slope</strong></td>
<td>9.575</td>
</tr>
<tr>
<td><strong>Intercept</strong></td>
<td>0.0263</td>
</tr>
<tr>
<td><strong>Correlation Coefficient</strong></td>
<td>0.9997</td>
</tr>
</tbody>
</table>
Figure (4) Linearity plot of Acetyl salicylic acid concentration versus absorbance

Figure (5) Plot of Acetyl salicylic acid concentration versus residuals
3.2 Precision

The method showed good repeatability, the relative standard deviation of five determinations performed in the same day was relatively small (0.951%) and less than the generally accepted value (2%), the method’s intermediate precision was also smaller than the accepted value of (3%) for intermediate precision. Table 2, 3 and 4 show the results of repeatability and intermediate precision

<table>
<thead>
<tr>
<th>Table 2. Repeatability results (day 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard</strong></td>
</tr>
<tr>
<td><strong>Sample No.</strong></td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td><strong>mean</strong></td>
</tr>
<tr>
<td><strong>Standard deviation</strong></td>
</tr>
<tr>
<td><strong>Relative standard deviation</strong></td>
</tr>
</tbody>
</table>
Table 3. Repeatability results (day 2)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
<th>% Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.39</td>
<td>100.58</td>
</tr>
<tr>
<td>2</td>
<td>0.388</td>
<td>100.06</td>
</tr>
<tr>
<td>3</td>
<td>0.39</td>
<td>100.58</td>
</tr>
<tr>
<td>4</td>
<td>0.389</td>
<td>100.32</td>
</tr>
<tr>
<td>5</td>
<td>0.399</td>
<td>102.90</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>100.89</td>
</tr>
</tbody>
</table>

Standard deviation: 1.14
Relative standard deviation: 1.135

Table 4. Intermediate precision results (day 1& 2)

<table>
<thead>
<tr>
<th>sample No.</th>
<th>Day</th>
<th>% content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>99.77</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>100.70</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>100.39</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>98.83</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>98.52</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>100.58</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>100.06</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>100.58</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>100.32</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>102.90</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>100.89</td>
</tr>
</tbody>
</table>

Standard deviation: 1.14
Relative standard deviation: 1.135
3.3 Recovery

The method showed good recovery results in the range of 100.72 – 101.53% of the labeled amount with very small relative standard deviation value (0.43%) as shown in Table 5.

Table 5. Recovery study data

<table>
<thead>
<tr>
<th>Added standard concentration (µg/ml)</th>
<th>Absorbance</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.372</td>
<td>0.421</td>
<td>0.418</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.494</td>
<td>0.541</td>
<td>0.537</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>0.608</td>
<td>0.662</td>
<td>0.652</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.723</td>
<td>0.776</td>
<td>0.773</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>0.832</td>
<td>0.893</td>
<td>0.882</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.966</td>
<td>1.022</td>
<td>1.021</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.0146</td>
<td>0.0149</td>
<td>0.0149</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>0.3730</td>
<td>0.4210</td>
<td>0.4159</td>
<td></td>
</tr>
<tr>
<td>Sample concentration (µg/ml)</td>
<td></td>
<td>25.3</td>
<td>28.0</td>
<td>27.5</td>
</tr>
<tr>
<td>Concentration recovered</td>
<td>25.48</td>
<td>28.23</td>
<td>27.92</td>
<td></td>
</tr>
<tr>
<td>% Recovery</td>
<td>100.72</td>
<td>100.83</td>
<td>101.53</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td>101.03</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td></td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>%Relative standard deviation</td>
<td></td>
<td></td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>
Figure (6) recovery plot of Acetyl salicylic acid absorbance versus concentration (Sample1)
Figure (7) recovery plot of Acetyl salicylic acid absorbance versus concentration (Sample2)
Figure (8) recovery plot of Acetyl salicylic acid absorbance versus concentration (Sample3)
3.4 Specificity

The method was proved to be specific for the determination of Acetyl salicylic acid in presence of its degradation product salicylic acid as the results of spiking Acetyl salicylic acid with varying amounts of salicylic acid ranging from 12.5% to 62.5% did not affect the assay results of Acetyl salicylic acid. The results are shown in Table 6.

**Table 6. Precision study data**

<table>
<thead>
<tr>
<th>Amount of Acetyl salicylic acid (mg)</th>
<th>Salicylic acid added (mg)</th>
<th>% added</th>
<th>Absorbance</th>
<th>% Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>0.4</td>
<td>12.5</td>
<td>0.5042</td>
<td>100.00</td>
</tr>
<tr>
<td>3.2</td>
<td>0.8</td>
<td>25.5</td>
<td>0.5038</td>
<td>99.92</td>
</tr>
<tr>
<td>3.2</td>
<td>2.0</td>
<td>62.5</td>
<td>0.5064</td>
<td>100.44</td>
</tr>
<tr>
<td>Standard</td>
<td></td>
<td></td>
<td>0.5042</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

The developed method was found to be specific, accurate and precise for the determination of Acetyl salicylic acid in presence of its major degradation product salicylic acid.
Recommendations

This method should be tried in the analysis of other systems where the major absorbing drug exists in equilibrium with its degradation products.
References

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(31) FDA (Food and Drug Administration), guidance for industry, Q3B impurities in new drug products 2006.
(41) Kamberi, M, Riley C and Sharon X (2004) "A validated, sensitive HPLC method for determination of trace impurities in acetaminophen drug substance " Journal of Pharmaceutical and Biomedical Analysis. 34, 123-128