Research Article

DETERMINATION OF TWO ANALGESICS (ACETYL SALICYLIC ACID AND ACETAMINOPHEN) BY A SINGLE CHROMOGENIC REAGENT

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ABSTRACT

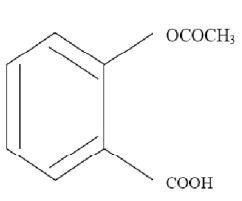
Ferric chloride reacts with acetyl salicylic acid and acetaminophen to give violet color having maxima at 520nm and a blue color having maxima at 560nm respectively. The reaction is selective for both the analgesics with 0.01mg/10ml as visual limit of quantitation and provides a basis for a new spectrophotometric determination. The color reaction obeys Beer's Law from 0.01mg/10ml to 2.5mg/10ml and the relative standard deviation is 0.85 for acetyl salicylic acid and 0.78 for acetaminophen respectively. The quantitative assessment of tolerable amounts of other drugs not interfering is also studied.

Keywords: Acetyl Salicylic Acid, Acetaminophen, Spectrophotometry, Ferric Chloride.

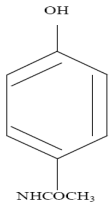
INTRODUCTION

The dire need of human beings is to relieve pain, therefore Salicylates and its derivatives have long been used for hypertension, headaches, mylgia, arthralgia etc. however its long term abuse can have direct effect on the central nervous system and can inhibit platelet aggregation thus producing prolonged bleeding[1]. Similarly in the analgesics of opioid origin side effects are vomiting, sweating, dizziness, nervous tremors, anxiety and seizures etc. [2]

A number of techniques have been employed for the determination of analgesics, specially salicylates, such as GC-MS[3], Copper hexacyanoferrata modified carbon paste electrode by cyclic voltametry[4], the analgesic activity in plant *scindapus officinalis*[5] and HPLC[6], all of which are lengthy and tedious procedures. In the spectrophotometric procedures usually a Visible and UV Spectrophotometry[7,8] and Derivative Spectrophotometry[9] methods are used, similarly the official methods involve UV, IR procedures which determined only one analgesic at a time.



Acetylsalicylic Acid



Acetaminophen (Paracetamol)
4-Hydroxyacetanalide

Figure 1: Structural Formulae of Acetylsalicylic Acid and Acetaminophen

During studies on drugs of abuse[10-13] it was found that Acetyl Salicylic Acid and Acetaminophen (Fig.1) react with a single chromogenic reagent, $FeCl_3$ in a neutral media to produce a violet and blue color having maximum absorbance at 520nm and 560nm respectively. The difference between the two absorbencies and their colors was used for the determination of both the analgesics. The reaction obeys Beer's Law and has 0.01mg/10ml as visual limit of quantitation. The present method is simple, accurate, precise and sensitive. Percentages of tolerable limits of other drugs which do not interfere are also studied.

MATERIALS AND METHODS

Instrument

Hitachi -II- 1100 spectrophotometer with 1 cm silica cells was used to measure the absorbance. A Beckman zerometric pH meter and graduated pipettes were employed.

Reagents

Analytical grade chemicals and doubly distilled water were used. Standard solution (w/v:1mg/ml) both of pure acetyl salicylic acid and acetaminophen were dissolved in 40 ml of distilled water separately in 100 ml measuring flask and the volume was made up to mark with distilled water to give a stock solution which was diluted further as required. 1% (w/v) Ferric Chloride solution was prepared by dissolving 1g of it in 100 ml distilled water.

Procedures

General Procedure

To an aliquot (1ml) of acetyl salicylic acid and acetaminophen containing 0.1 to 2.5mg/10 ml was added 2 ml of 1% ferric chloride and the volume was made up to 10ml separately with distilled water. After shaking the resulting absorbance of violet color (for acetyl salicylic acid) and blue color (for acetaminophen) were measured at 520nm and 560 nm respectively, employing only the reagent as a blank.

The experiment was repeated with different concentrations of both the analgesics and a calibration

curve was prepared Fig.2, %RSD is shown in Table 1. The color reaction obeys Beer's Law from 0.01 mg/10ml to 2.5mg/10ml of both the analgesics and the results are reported in mg /10ml for both the drugs.

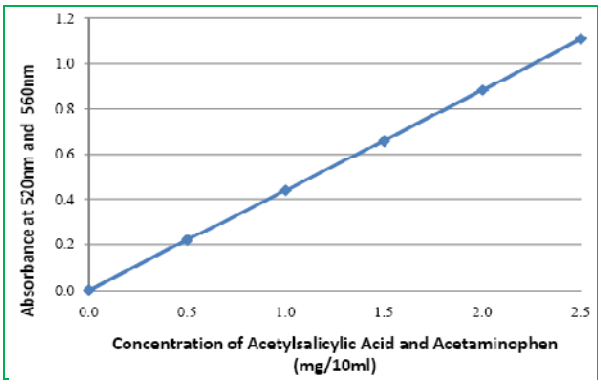


Figure 2: Calibration Curve for Acetylsalicylic and Acetaminophen

Procedure for studying the interfering compounds

Interferences from various drugs were studied at low concentrations because higher concentrations made the procedure uneconomical. Thus to an aliquot containing 1mg/ml organic compounds (1mg/ml) having similar action and common interferences in the analysis of analgesics, were added individually until the solution showed the same (±0.01) absorbance as that of pure acetyl salicylic acid and acetaminophen solutions under the experimental conditions described above. The value was calculated as the percentage of the organic compounds with respect to the amount of both the analgesics separately.

Procedure for the determination in pharmaceutical preparations

Tablets

One tablet containing 300mg of acetyl salicylic acid and one tablet containing 500mg of acetaminophen were powdered, weighed and dissolved in distilled water and filtered separately. The filtrate was diluted to get 1mg/ml solution of both the analgesics. An aliquot containing 0.01mg to 2.5mg/10 ml was taken. The procedure was followed as described above and the absorbance was measured at 520nm for acetyl salicylic acid and 560nm for acetaminophen. The quantity per tablet was calculated from the calibration graph.

Syrup

Syrup containing 120mg/50 ml of acetaminophen was diluted with distilled water, filtered and 1mg/ml solution of acetaminophen was prepared. An aliquot containing 0.01mg to 2.5mg/10ml was taken, the above procedure was followed and the absorbance of the blue color was measured at 560nm. The quantity of acetaminophen per 50ml of the syrup was calculated from the standard calibration graph.

RESULTS AND DISCUSSION

Absorption spectrum of the colored complex

Both the analgesics i.e. acetyl salicylic acid and acetaminophen react with ferric chloride in the neutral media, (no heat required) to give violet and blue colored complexes respectively, the absorption maxima of the complexes under optimum conditions are 520nm violet, 560nm blue (Fig. 3).

Table 1: Determination of Acetyl Salicylic Acid and Acetaminophen from Pure Solution

Acetyl Salicylic Acid Taken (mg/10ml)	Acetyl Salicylic Acid Found* (mg/10ml)	RSD %	Acetaminophen Taken (mg/10ml)	Acetaminophen Found* (mg/10ml)	RSD %
0.1	0.092	0.85	0.1	0.09	0.78
0.2	0.19	0.52	0.2	0.18	0.34
0.3	0.29	0.34	0.3	0.29	0.25
0.4	0.39	0.25	0.4	0.39	0.20
0.5	0.49	0.20	0.5	0.49	0.10
1.0	0.98	0.10	1.0	0.95	0.06
1.5	1.47	0.06	1.5	1.45	0.05
2.0	1.95	0.05	2.0	1.95	0.04
2.5	2.45	0.02	2.5	2.45	0.03

* Every reading is a replicate of five independent measurements

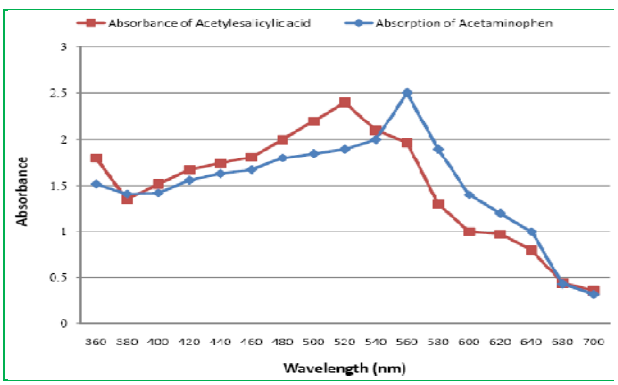


Figure 3: Absorption Spectra of Acetylsalicylic acid and Acetaminophen

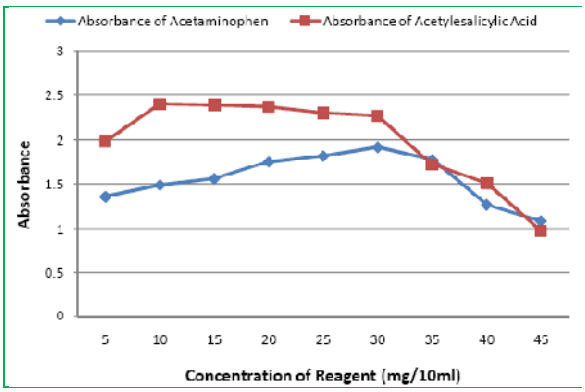


Figure 4: Effect of Ferric Chloride on the Colored Complex of Acetylsalicylic Acid and Acetaminophen

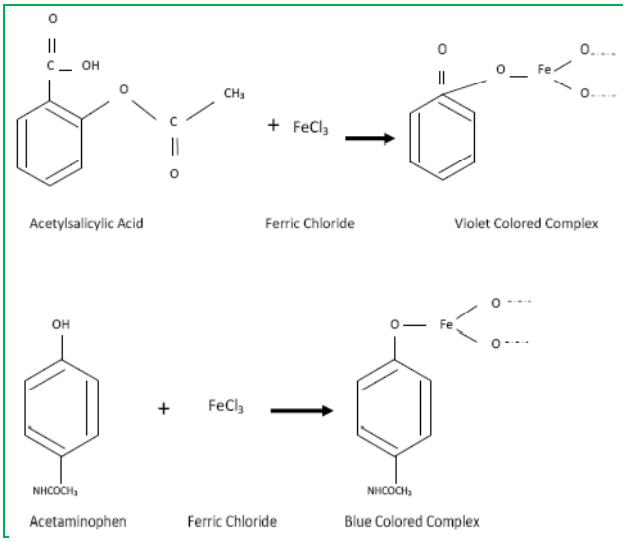


Figure 5: Probable Mechanism

Effect of color producing reagent

Ferric chloride 3ml (1% w/v) was used as a color producing reagent. It was found that 10mg/10ml and 30mg/10ml of both the analgesics Acetyl Salicylic Acid and Acetaminophen gave maximum color respectively (Fig. 4). The color reaction is stable for more than 24 hours and Beer's Law is obeyed from 0.01mg/10ml to 2.5mg/10ml respectively. The color did not diminish during measurement. Other oxidizing reagents such as Sodium Nitrate, Hydrogen Peroxide, Sodium Nitrite, Potassium Dichromate, Potassium Iodate and Potassium Iodide were tested for production of color and it was observed that none of them reacted with both the analgesics to give a colored complex. Neither solvent nor temperature is required to produce the colors. Above and below these concentrations the colors intensity diminished and the color became unstable. The probable mechanism of the color reaction is that ferric chloride forms a metal ligand complex between both the analgesics, as shown in (Fig.5) i.e. hydrogen is removed from the carboxylic acid group and the ferric ion is attached on to the site as Fe (III) ion. Ferric (III) ion has three other sites of attachment that of the other two oxygen atoms, which on the other hand combines with another molecule of acetyl salicylic acid and a new metal ligand, is formed. Whereas in the case of acetaminophen there are two reactive groups, hydroxyl and a carbonyl group. Out of the two sites the hydroxyl group is more reactive than the carbonyl group, so the hydroxyl group reacts with the ferric ion to form the colored complex ligand having a maximum at 560nm.

Effect of organic solvent

Different organic solvents, such as, alcohol, chloroform, benzene, hexane, methanol, acetone, and methyl ketone were tested for color extraction and for stability but none was effective. The color was stable for more than 24h without the addition of any solvent. The complex developed in water without the formation of any emulsion.

Sensitivity, precision and accuracy

The results for the determination of pure acetyl salicylic acid and acetaminophen are shown in Tables 1 and 2 which show the sensitivity, validity and repeatability of the method. It is also precise and accurate as the amount taken from identical samples is known and the amount found by the above procedure does not exceed the relative standard deviation, 0.85 and 0.78 for Acetyl Salicylic Acid and Acetaminophen respectively which is the replicate of five determinations (cf Table 1). There are no interferences of synthesis by-products of the present color reaction. The optimization has been done at lower analyte concentration. The calibration graph is linear in the range of 0.01 to 2.5mg/10ml. The apparent molar absorptivity calculated was 0.1436×10^4 for acetylsalicylic acid and 0.1729×10^4 for acetaminophen and the regression equation[14] was calculated by the method of least square from twelve points, each of which was the average of four determinations. The correlation between absorbance and concentration was 0.999 in terms of correlation coefficient (r) for both the analgesics (cf Table 2).

Table 2: Optical Characteristics, Precision and Accuracy of the Proposed Method

Parameters	Acetyl Salicylic Acid Values	Acetaminophen Values
λ_{max} (nm)	520	560
Beer's Law Limit (mg/10ml, C)	0.01-2.5	0.01-2.5
Molar Absorptivity ($mol^{-1}cm^{-1}$)	0.1729×10^4	0.1436×10^4
Regression equation Y*		
Slope (b)	0.96	0.95
Intercept (a)	0.001818	0.00354
Correlation coefficient (r)	0.999	0.9998
Relative standard deviation (RSD)**(%)	0.85	0.78
%Range of error (Confidence Limits) at 95% confidence level	99.8±0.002	499.94±0.04

*Y= a+ bC, where C is the concentration of analytic (mg/10mL) and Y is the absorbance unit; **Calculated from five determinations.

Table 3: Quantitative Assessment of Tolerable Amounts of Other Drugs

Drugs	Acetyl Salicylic Acid Tolerable amount not interfering* (%)	Acetaminophen Tolerable amount not interfering* (%)
Carvidilol	100	200
Tarazosine	100	200
Noproxime	100	150
Diclofenic sodium	150	300
Ibuprofen	100	150
Diclofenic Potassium	100	300
Indomethacin	150	100
Triprolidine HCl	200	250
Amitriptiyline HCl	50	100
Methyl dopa	100	100
Acetic anhydride	250	250
Trifluoperazine HCl	100	100
Cyclizine HCl	150	200
Mefenmic acid	200	200
Thiosidazine HCl	100	100
Sorbitol	300	300

*The value is the percentage of the drug with respect to 1mg/ml of the analgesics that causes ±0.01 change in absorbance

Interferences

The quantitative assessment of different organic compounds (W/V) under the experimental conditions is given in Table 3. Various amounts of diverse interfering compounds having similar actions as analgesics were added to a fixed amount of acetyl salicylic acid and acetaminophen (1mg/10ml) of both the analgesics and the recommended procedure for the spectrophotometric determination was followed.

Application

The proposed method is successfully applied for the quality control of pure analgesics and in their pharmaceutical dosage form as shown in Table 4 and 5. The excipients and the added color agents in the formulations did not interfere with the analysis.

Table 4: Determination of Acetylsalicylic Acid from Pharmaceutical Preparations

Drug	Trade Name	Pharmaceutical Preparations	Amount present manufacturer specification (mg)	Amount found by present method	Amount found by standard B.P procedure (mg)	Recovery %
Acetylsalicylic Acid	Adalgin (Adamjee Pharmaceutical Pakistan)	Tablets	300	299.95	300.01	99.98
	Aspro (Reckitt Benkiser Pakistan)	Tablets	300	300	300	100
	Emiprin (Glaxo, Pakistan)	Tablets	300	299.93	309.99	99.99
	(Smith Kline)	Tablets	300	299.94	300	9.98
	Eprin (Epoch Pharmaceutical)	Tablets	300	299.96	300.02	99.98
	Dispirin (Reckitt Benkiser, Pakistan)	Tablets Tablets	300 100	300.1 100	300.01 100	100.03 100
	Loprin (Highnoon labs, LHR. Pakistan)	Tablet	75	75.1	75.01	100.03

Table 5: Determination of Acetaminophen from Pharmaceutical Preparations

Drug	Trade Name	Pharmaceutical Preparations	Amount present manufacturer's specification (mg)	Amount found by present method (mg)	Amount found by standard B.P procedure (mg)	Recovery %
Acetaminophen	Acetosol (Shaigan Pharmaceutics)	Tablets	500	500	500.01	100
	(Amdol (Hermman Pharmaceutics)	Tablets	500	499.98	500	99.99
	(Amdol (Hermman Pharmaceutics)	Syrup	120/50ml	119.95	500.02	99.95
	Askprol (Askari Pharmaceutics)	Tablets	500	500.01	500.01	100.02
	Barbidol (Wilshill Ltd.)	Tablets	500	500.02	499.99	100.04
	Calpol (Glaxo, Pakistan)	Syrup	120/50ml	120	120	100

CONCLUSION

Determination of two or more compounds in the same sample without previous separation is a basic analytical problem. In the present study the resolution of the binary mixture of two analgesics can be determined by a very simple sensitive, precise, accurate and less time consuming spectrophotometric method. The statistical analysis is in good agreement with British Pharmacopeia 2007. The color reaction is specific for both salicylic acid and acetaminophen. The method can be successfully applied to the trace determination of both the analgesics in pure as well as in the pharmaceutical preparations. The color reaction has 0.01mg/10ml as visual identification limit. Other drugs having similar actions do not interfere.

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