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COMPARISON OF SPECTROPHOTOMETRIC AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHODS IN THE BIOAVAILABILITY STUDIES OF ASPIRIN AND SALICYLIC ACID IN HEALTHY HUMAN VOLUNTEERS

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Abstract
The aim of the work was to develop a simple, rapid and accurate multiple component spectrophotometric method for the determination of aspirin and its major metabolite, salicylic acid, in plasma and the results obtained compared with the results of the same analysis using HPLC method. With HPLC, the drugs aspirin, salicylic acid and paracetamol (as internal standard) were eluted with 10µm reversed phase C_{18}-support column at ambient temperature with a mobile phase consisting of methanol: 5% acetic acid (25:75) adjusted to pH 3.45 with glacial acetic acid, at a flow rate of 1.5ml/min with U.V detection at 280nm. Each analysis required no longer than 10min. Quantitation was achieved by measurement of the peak-height ratio and the relative and absolute recoveries varied from 90 to 98%. For U.V. spectrophotometric analysis, Beer's law plots were prepared using standard solutions of each of the salicylates (aspirin and salicylic acid) in 0.1M HCl and plasma. Extraction recoveries in plasma and 0.1M HCl (as control) were developed and the relative and absolute recoveries varied from 90 to 98%. The methods were applied for the determination of the pharmacokinetic parameters of aspirin and its major metabolite salicylic acid. Comparing the bioavailability of the drug under the two analytical methods employed in the study, it was observed that higher concentrations were observed with HPLC than U.V. Spectrophotometric method. However, the results obtained from these two analytical methods correlate with each other with the degree of probability ($0.05<P<0.1$) using student's t-test. In the absence of high-performance liquid chromatography, U.V. spectrophotometric method could also be used in the bioavailability studies of aspirin and its major metabolite salicylic acid with measurable degree of accuracy and sensitivity.

Keywords: Comparison of Analytical Methods, Bioavailability of Aspirin, Human.

INTRODUCTION

Aspirin is a non steroidal anti-inflammatory and anti-pyretic agent used in degenerative joint disease, rheumatoid arthritis and allied conditions. (1)

Various methods have been reported for quantifying aspirin and its major metabolite in different biologic media. These include the standard colorimetric (2) and fluorimetric (3) methods and the more modern analysis by gas-liquid chromatography (4) and high performance liquid chromatography (HPLC) (5).

HPLC has always been the analytical technique of choice over many principally because of its speed, high specificity and selectivity for separating very closely related compounds. However in developing

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countries like Nigeria, procurement and maintenance of these highly fragile and expensive instruments have posed a number of problems to teaching and research in the recent past. Poor funding on education, epileptic water and power supplies to our laboratories, lack of spare parts and experts to service and maintain these instruments have further complicated matters to the point that most of the instruments already procured have packed up and no enough funds to procure new ones.

To this end, the present study has reported an alternative analytical technique for the determination of aspirin and its major metabolite, salicylic acid, in plasma using a simple, rapid and accurate multiple component spectrophotometric method and the results obtained compared with the results of the same analysis using high performance liquid chromatographic method as the standard.

MATERIALS AND METHODS

Materials: All reagents and solvents used are of analytical grade. Standard aspirin and salicylic acid powders were obtained from M & B Lab. Chemicals. The internal standard paracetamol was obtained from Abbott Labs. (North Chicago, IL, U.S.A). Polfa aspirin tablets with batch No. 50790 were obtained from Polfa (Nig). Limited.

Instrumentation: HPLC. Waters 204 HPLC model equipped with 441 model U.V. detector fitted with 254mm filter model U6K septumless injector and SE 120 model was used. The column used for the chromatographic separation was a μ-Bondapak radial pack cartridge, polyethylene 15cm x 8.0mm I.D packed with 10μm reversed phase C_{18} – support, waters part No. 85721, (Waters Associated, Inc; Wilford).

UV-Spectrophotometry. SP8-100 ultraviolet spectrophotometer (PVE-UNICAM LTD., York Street, Cambridge, England CBI 2PX). Erweka dissolution apparatus and accessories, Erweka Apptebau G.M.B.H 6056. Heusenstamm Kr. Offenbuch – Main. Analytical balance, type H35 AR Mettler, Gallenhamp, Silica cuvettes B.S. 3875.3 10mm F.O.

Methods: Identification tests, disintegration and dissolution rate tests and assay for content of active ingredient in the aspirin tablets were carried out as per the BP 1993 procedure.

Simultaneous Determination of Aspirin and Salicylic acid in Plasma Using Multiple Component Spectrophotometric Method: This was done by determining the absorption spectra of the two pure compounds (Standard aspirin and salicylic acid solutions). This led to the selection of two analytical wavelengths in which absorption by the compounds is maximal at these wavelengths, with the absorptivity for one compound being greater than the other at one wavelength and less at the other.

The next step in the analysis was the making of Beer's Law plots, using solutions of the pure substances for each compound at each wavelength. This gave four Beer's Law plots from which were calculated four absorptivity (slope = ab, where a= absorptivity and b= internal path length of the sample cell) which may be symbolized as a_1^{ASA} a_2^{SA} a_2^{SA} a_2^{ASA} with the superscript indicating the compound and subscript the wavelength. Any solution containing aspirin and salicylic acid was analyzed by measuring the absorbance of the solution at the two wavelengths.

Assuming that the absorbances of the mixture are equal to the sum of the absorbances of the components of the mixture, it follows that if A1 and A2 are the absorbances of the mixture at the two wavelengths, then;

$$A_1 = {}^{ASA}A_1 + {}^{ASA}A_1$$

$$A_2 = {}^{ASA}A_2 + {}^{SA}A_2$$

$$A_1 = {}^{ASA}a_1\ bc^{ASA} + {}^{SA}a_1\ bc^{SA}$$

$$A_2 = {}^{ASA}a_2\ bc^{ASA} + {}^{SA}a_2\ bc^{SA}$$

From the above equations, the concentrations of the components of the solution mixture were analyzed.

Simultaneous Determination of Aspirin and Salicylic Acid in Plasma Using High Performance Liquid Chromatograph (HPLC):

The chromatographic condition for separation of the drug and its metabolite were set as follows:- mobile phase, methanol: 5% acetic acid (25:75), pH 3.45, flow rate 1.5ml/min., recorder chart speed 0.5cm/min., detection wavelength 280nm, injection volume, 15µl, sensitivity 0.02 aufs. Under the described chromatographic conditions, the mean retention times were 3.0, 5.0, and 7.0 minutes for paracetamol (internal standard), salicylic acid and aspirin respectively.

Preparation of Standard Solutions (Beer's Law plots):

50mg of each of the salicylates (ASA and SA) were accurately weighed and dissolved in 100ml methanol to yield a stock solution of 500µg/ml. This stock solution was further diluted to give working standards ranging from 1 to 100 µg/ml for aspirin; and 1 to 120µg/ml for salicylic acid.

Plasma calibration graph was prepared by spiking 1ml blank plasma sample with working standards ranging from 1 to 120µg/ml for salicylic acid and 1 to l00µg/ml for aspirin. Each of these concentrations after extraction were run in the UV spectrophotometer and the absorbances noted at 276nm and 231nm respectively. A graph of concentration against absorbance at each wavelength was plotted.

Extraction Procedure (UV)

Blank plasma (1ml) spiked with 1ml working standards was mixed with a 2ml aliquot of 0.05M HCL in a glass-stoppered tube. After the addition of 1Oml ethylacetate, the drugs were extracted by shaking the tubes gently for 15 minutes on a mechanical shaker followed by centrifugation for 15 minutes at 5000g. Exactly 5ml of the supernatant were transferred in a glass tube, which was placed in a water bath at 40^0C and the solvent was evaporated under a gentle stream of oxygen free nitrogen. The residue was reconstituted in 5ml methanol. The absorbance of the clear solution was measured at 276nm and 231nm respectively for each of the solutions. A graph of absorbance against concentration was then plotted for each wavelength. The levels of aspirin and salicylic acid were derived from these graphs. The above extraction was repeated with 10 and 20µg/ml of aspirin and salicylic acid without plasma and percent extraction recoveries of aspirin and salicylic acid at 231nm and 276nm calculated.

Preparation of standard solutions and calibration graphs (HPLC)

Working solutions of appropriate concentrations were prepared every week by dilutions of the stock solutions with methanol.

Plasma calibration graph was prepared by spiking blank plasma sample (200µl) with working standards ranging from 1 to 120µg/ml for salicylic acid; 1 to 100µg/ml for aspirin and 20µg/ml for paracetamol as internal standard. The samples were processed as described below in the extraction procedure for HPLC.

Standard graphs were constructed by plotting the peak height ratio of aspirin to the internal standard against the drug concentration in each standard and also peak height ratio of salicylic acid to the internal standard against the drug concentration in each standard. The level of the drugs in an unknown plasma sample was derived from these graphs.

Extraction Procedure (HPLC)

Aliquots of blank plasma (200µl) were mixed with 20µl of a 30% perchloric acid solution containing the internal standard paracetamol (20µg/ml) in 1.5ml polypropylene Eppendort microtest tubes; followed by a pinch of sodium chloride salt and 200µl methanol. The mixture was then vortexed for 2 mins and centrifuged for 4 mins. A 20µl sample of the clear supernatant was injected into the chromatogram. The above extraction procedure was repeated with 40, 60, 80µg/ml aspirin and salicylic acid concentrations and 20µg/ml of paracetamol without plasma and percent extraction recoveries of aspirin, salicylic acid and paracetamol (20µg/ml) calculated.

In vivo Study:

Six healthy male volunteers participated in the study. The average age and weight of the volunteers were 22.3 years and 61.3kg respectively. The volunteers were clinically certified fit for the study and were asked to refrain from taking any drug for at least 2 weeks before the commencement of the study. They were all non-smokers and non-alcoholics. Each volunteer was given 600mg dose of aspirin tablets with about 100ml water after overnight fast. Food was withheld for another 2hrs after ingestion. Blood samples (5ml) were immediately withdrawn prior to ingestion and then at 0.5, 1,2,3,4,5 and 6 hr intervals after ingestion via an indwelling canula. The blood samples were placed in heparinised bottles, centrifuged for about 15 minutes at 2000g and the plasma stored at -2^0C pending analysis.

RESULTS AND DISCUSSION:

Assay for content of active ingredient, disintegration and dissolution rate tests of the brand of aspirin tablets examined showed conformity with the BP 1993 requirements (see tables I and II). Linear calibration curves for aspirin and salicylic acid with good correlation coefficients were obtained using both HPLC and UV spectroscopic techniques.

Fig. 1 shows HPLC chromotogram of the extracted plasma sample of aspirin at the maximum concentration. Aspirin can be seen to be separated well from its metabolite, salicylic acid and internal standard paracetamol with retention times of 3.0, 5.0 and 7.0 minutes for paracetamol (internal standard), salicylic acid and aspirin respectively.

The mean estimated plasma pharmacokinetic parameters under the two analytical methods (HPLC and UV spectrophotometric) for both aspirin and its major metabolic product, salicylic acid, are shown in tables III and IV respectively.

Table 1: Chemical assessment of aspirin and salicylic acid standard and content of aspirin and aspirin tablets as per BP 1988 requirement.

Sample	Content (%)
Aspirin Powder	99.90
Salicylic acid powder	104.50
Aspirin tablets	100.30

Table II: Disintegration and Dissolution rates of Aspirin tablets as per BP 1988 requirements

Sample	Mean Disintegration rate (6 tablets) (min)	Mean (%) release rate at 45 min (5 tablets)
Aspirin tablets	2.92±0.42	100.0±0.22

Table III: Determination of the mean Plasma Pharmacokinetic Parameters of Aspirin after a Single Oral Dose (600mg) of Aspirin to Six Human Volunteers using High Performance Liquid Chromatographic (HPLC) and UV Spectrophotometric Methods.

Parameters	HPLC	UV-Spectrophotometer
t_{max} (hr)	0.50±0.19	0.50±0.17
C_{max} (μg/ml)	11.28±0.71	10.04±0.11
$t_{½}$ α (hr)	1.42±0.82	1.04±0.12
K_a (hr^{-1})	1.043±0.44	1.098±0.70
K_{el} (hr^{-1})	0.637±0.21	0.638±0.17
$AUC_{(0-6h)}$ (μg/ml.hr)	16.98±0.21	14.03±0.11

Table IV: Determination of the Mean Plasma Pharmacokinetic Parameter of Salicylic Acid after a Single Oral Dose (600mg) of Aspirin to Six Human Volunteers, using High-Performance Liquid Chromatographic (HPLC) and U.V. Spectrophotometric Methods.

Parameters	HPLC	UV-Spectrophotometer
t_{max} (hr)	2.0±0.72	2.0±0.18
C_{max} (μg/ml)	65.05±0.29	43.84±0.21
K_{el} (hr^{-1})	6.09±0.08	7.37±0.29
$AUC_{(0-6h)}$ (μg/ml.hr)	183.31±0.43	171.59±0.07

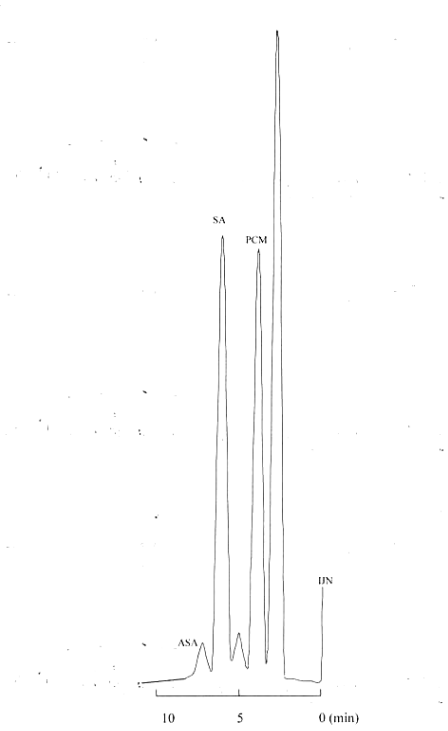

FIG. 3: HPLC Chromatogram of Plasma Aspirin and Salicylic acid at time of maximum Concentration (C_{max}) and Internal Standard (15μg/ml).

PCM = Paracetamol; SA = Salicylic Acid; ASA = Acetyl salicylic acid.

Fig. 1

Comparing the mean plasma pharmacokinetic data for aspirin and salicylic acid under the two analytical methods employed in the study, slightly higher plasma concentrations were observed with HPLC than UV -spectrophotometric method. For instance, the C_{max} for aspirin under HPLC method was 11.28±0.71μg/ml and with U.V. spectrophotometric method was 10.04±0.11μg/ml ($P<0.05$) See table III. Similarly the $AUC_{(0\text{-}6hr)}$, $t_{½}\alpha$ K_a, K_{el} and t_{max} of aspirin under the two analytical methods indicated little or no difference in the calculated pharmacokinetic parameters ($P<0.05$). The results obtained from these two analytical methods correlate with each other with the degree of probability ($0.05<P<0.1$) using student t-test.

The observed slightly higher plasma concentrations of aspirin with HPLC compared to UV -spectrophotometric method could be as a result of its higher sensitivity for assay of drugs in biological fluids and higher specificity and selectivity for separating very closely related compounds. Also, the presence of other metabolites may have interfered with the UV -spectrophotometric absorption of either aspirin or salicylic acid especially those metabolites which absorb at the same wavelength.

CONCLUSION

In the analysis of aspirin and its metabolite in plasma, HPLC is the most ideal method compared with the UV spectrophotometric method because of its higher sensitivity and reliability. But in the absence of HPLC, UV spectrophotometry could also be used as an alternative method following the method employed in this study.

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