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Development and validation of a reversed phase High Performance Liquid Chromatography method for content evaluation of some brands of paracetamol tablets sold in Abuja, Nigeria

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Abstract
A simple, rapid, accurate and economical isocratic Reversed Phase High Performance Liquid Chromatography (RP-HPLC) method was developed, validated and used for the evaluation of content of different brands of paracetamol tablets. The method was validated according to ICH guidelines and may be adopted for the routine analysis of paracetamol in dosage forms both as single or combined formulation. Twelve brands of paracetamol tablets were randomly purchased from pharmacy stores around Abuja, the Federal Capital Territory (FCT) of Nigeria and coded accordingly. Samples were analyzed using a validated isocratic RP-HPLC method with UV detection at 254 nm at ambient temperature. Analysis time was 6 minutes. The inter and intraday precision coefficient of variance for 2 and 8 µg/ml were less than 5% and the percentage recovery was more than 90%. Percentage contents ranged between 93.03- 126.43% (±0.81-11.12). 41.7% of the assessed brands passed while 58.3% failed with the values being either above or below the BP specification of 95-105% for percentage content of paracetamol in tablets.

Keywords: Paracetamol; Reversed Phase HPLC; Routine Analysis; Pharmacy Stores

INTRODUCTION

Paracetamol, N-(4-hydroxyphenyl) acetamide, is a para-aminophenol derivative. It is a non-opiate, non-salicylate, centrally and peripherally acting analgesic agent. Paracetamol is available in different dosage forms: tablet, capsules, drops, elixirs, suspension and suppositories. It is a white, odorless crystalline powder with a bitter taste. It is also commonly known as acetaminophen. Paracetamol tablets are listed among the essential drugs selected for the healthcare delivery system in Nigeria.

Paracetamol belongs to the class of Over-The-Counter (OTC) drugs. It is among the most consumed non-narcotic analgesic-antipyretic agent. The drug is used for temporary relief of mild to moderate pain such as headache, myalgia and postpartum pain. It is also used as suitable alternatives for the management of patients with some degree of haemophilia, osteoarthritis (OA), peptic ulcer and those who cannot be administered

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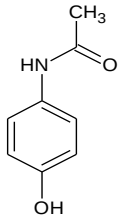
non-steroidal anti-inflammatory drugs (NSAID) [1,2]. Furthermore, paracetamol was recommended by the American College of Rheumatology (ACR) as the first-line treatment for pain associated with OA [3]. Fukushima *et al.* in a study demonstrated the gastro-protective effects of paracetamol in NSAID-induced gastric mucosal damage [4]. In a 2006 Cochrane review involving the pooled data of five randomized controlled trials that compared paracetamol with placebo, paracetamol demonstrated a modest improvement in pain relief compared with placebo [1]. As a result of the Nigerian government's policy of generic prescribing, the liberalization of trade and import laws, and the ever-increasing number of pharmaceutical industries, a wide range of paracetamol products appear on the Nigerian market. According to the Nigeria National Drugs Policy, only drugs conforming to nationally accepted and/or internationally recognized quality standards shall be permitted to be procured and distributed in the country. Any study therefore designed to monitor and improve the quality evaluation of pharmaceutical products both at the time of registration and post-market is very essential to the policy and technical guidelines of drug regulatory authorities such as the National Food and Drug administration control (NAFDAC). Such a study also benefits the Nigerian Health Service in the sense that procurement staff, prescribers, dispensers and patients have access to high-quality and efficacious drug products. Pharmaceutical industries may also be motivated to establish simple analytical procedures for both in-process and finished product evaluations. Hence it becomes imperative to evolve a system that is cost effective and selective for paracetamol in the bulk powder, dosage forms for easier efficacious drug products.

Several papers in the literature described the assay of paracetamol and its combination in pharmaceuticals or biological fluids with different methods most of which can be complicated vis-à-vis routine analysis and quality assessment. We had previously reported on the quality control assessment and post marketing surveillance of other heavily consumed OTC drug such as Vitamin C. Thus, this part of an on-going study on impact of quality control regulation and post-marketing surveillance with regards to compliance with official compendia for the OTC drugs.



N-(4-hydroxyphenyl) acetamide (Paracetamol)

The objectives of this study were to develop and revalidate a simple, reliable and accurate HPLC method for paracetamol and use this method to investigate the percentage content of different brands of paracetamol sold in some pharmacy stores of the Federal Capital Territory (FCT) of Nigeria so as ascertain the conformity with official specification and demonstrate the impact of post-marketing surveillance on one of the most consumed OTC drugs in the drug market.

EXPERIMENTAL

Materials: Agilent 1200 series HPLC system with Ultra-Violet/ Diode Array (UV/DAD) detector, a binary Pump and degasser was used for the analysis. A C18 Column (250 mm x 4.6 ID, 5µm) was employed for the analysis. Glassware, aluminum foil, precision pipette, mortar and pestle, membrane filters (0.45µm; 47mm and 0.2µm; 13mm) for solvent and sample filtration respectively. HPLC grade methanol (Sigma-Aldrich), 0.1 N NaOH (BDH), distilled water. Paracetamol Reference (Chemcon GmbH, Germany). Twelve different brands of paracetamol tablets were purchased randomly from different pharmacy stores at different

locations in the Federal Capital Territory (FCT) of Nigeria.

Sample collection: Twelve brands of paracetamol tablets were randomly purchased from different pharmacy stores in and around the Federal Capital Territory (FCT). The drugs were coded accordingly before analysis commenced and detailed information provided by manufacturers was recorded and the packaging also examined physically for proper packaging and labeling.

Mobile phase: The Mobile phase composition for the assay was water: methanol (60:40) V/V and the diluent was distilled water. Wavelength was 254 nm and the flow rate for the mobile phase was 1 ml/min with a run length of 6 minutes.

Visual examination of sample: This involved an inspection of the following parameters; shape (circular, oval, flat sides, other), uniformity of shape, uniformity of colour, no physical damage (cracks, breaks, abrasions, sticky), other observations (no foreign contaminant, dirty marks, proper seal). The basic tests conducted were based on WHO categorization of genuine, substandard and counterfeit [5].

Weight uniformity test: Ten tablets in each group of tablets were weighed individually (x), collectively and the weight recorded (Σx). The mean, of each group was then calculated and 5% of the mean was calculated. The weight variation was then calculated as Mean ± 5% of the mean. If two tablets out of 10 are outside the range, the tablets were considered to have failed the weight variation test. The tablets were then crushed into powder and stored in airtight containers [6].

Preparation of standard stock solutions: The stock solution of paracetamol standard (1mg/ml) was prepared by dissolving 10 mg paracetamol in 10ml solution of distilled water in a 10ml volumetric flask with vigorous shaking and ultra-sonicated for 10 minutes; the resulting solution was filtered through 0.2µm, 13mm diameter membrane filter. A working standard solution of 100µg/ml was prepared from the stock of 1mg/ml. Different concentrations were prepared from the working standard solution for calibration curve.

Preparation of sample: Ten (10) tablets of each brand of 500 mg paracetamol each were weighed and crushed finely into powder. An equivalent weight of 10 mg was accurately weighed and transferred into a 10-ml volumetric flask, dissolved in double distilled water and sonicated for 10 min. The resulting solution was allowed to stands for some time, and filtered through 0.2 µm membrane filter resulting in a concentration of 1 mg/ml stock solution. From this solution 1 ml was taken and diluted to 10 ml with the mobile phase to give a working solution of 100µg/ml. A concentration of 7.5µg/ml was prepared and 20µl injected unto the column in triplicate. This procedure was repeated for each of the brands and average peak areas for the various samples were calculated from the chromatograms. The actual concentration of paracetamol in each of the samples analyzed was interpolated from a calibration curve using the average peak area.

Calibration curve: Different concentrations of 2, 4, 6, 8, 10 and 14µg/ml were prepared from the working standard of 100µg/ml of the standard. 20 µl of each was introduced unto the column in triplicate. Concentrations that were not detected proportionally defined the limits of detection under the given set of experimental conditions.

Interday and intraday precision: The interday and intraday variation was carried out using two concentrations in accordance to ICH guide lines [7].

RESULTS:

A validated isocratic HPLC method was used for the analysis and the calibration curve was

linear over the concentration range of 2µg/ml to 14µg/ml with a correlation coefficient of 0.9991, a typical chromatogram of paracetamol is as shown in Figure 1a and b. While Figure 2 showed the calibration curve of the reference standard. Table 1 showed the percentage content of the different brands of paracetamol tablets and relative standard deviation for the analysis carried.

DISCUSSION

Twelve brands of the paracetamol 500mg tablets were analyzed using some quality control parameters such as visual inspection, weight variation, method validation and percentage content analysis. Visual inspection of a drug product and its packaging is usually meant to check the authenticity of that product. The product labels for all the brands examined had manufacturing date, expiring date and NAFDAC number.

Weight variation test is a very important quality control parameter because it bears a direct relationship with the content uniformity of a drug product. A tablet for instance, is designed to contain a specific amount of a drug so it is necessary to ensure that the exact amount is packaged as stated in the label claim. [8].

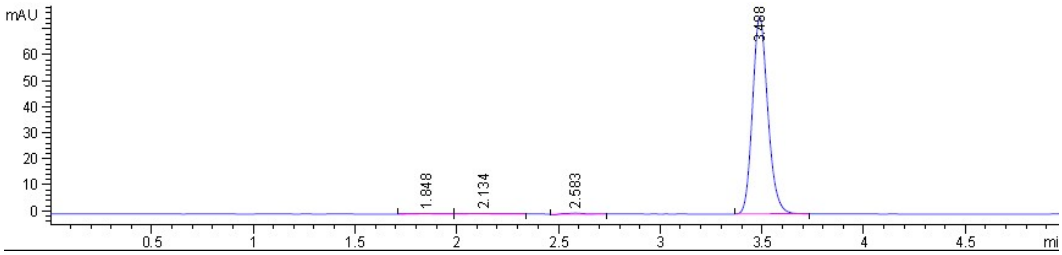


Figure 1a: Chromatogram for paracetamol standard and sample at 254nm

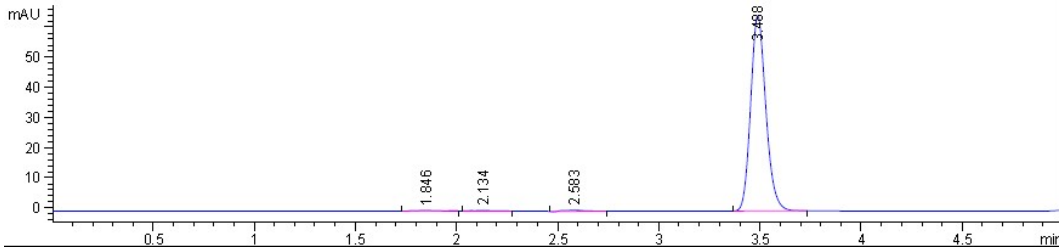


Figure 1b: Chromatogram for paracetamol sample at 254nm

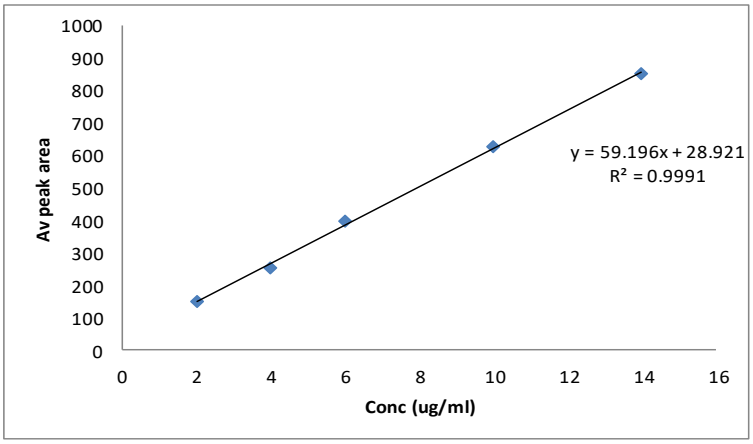


Figure 2: Calibration curve for paracetamol standard using the HPLC.

Table 1: Intraday and Interday Precision

Nominal concn. (µg/ml)	Calculated concn. (µg/ml)	Percentage recovery (%)	Mean of calculated conc. (µg/ml) ±SD	% CV
		Intraday Precision		
2	1.85	92.5	1.81±0.05	2.64
2	1.83	91.5		
2	1.74	87.0		
8	8.03	100.37		
8	7.80	97.50	7.94±0.25	3.15
8	7.72	96.50		
		Interday Precision		
2	1.85	92.50		
2	1.83	91.50		
2	1.81	90.50	1.84±0.02	1.09
2	1.85	92.50		
2	1.86	93.0		
8	8.01	100.13		
8	7.80	97.50		
8	7.72	96.50	7.70±0.21	2.73
8	7.58	94.74		
8	7.38	92.20		

Table 2: weight variation and coefficient variation

Sample Code	Weight Mean (mg) ±SD	% CV
A	552.00±7.04	1.2745
B	575.60±13.57	2.3582
C	587.90±5.45	0.9263
D	659.60±12.50	1.8954
E	581.20±0.03	0.0052
F	570.40±0.03	0.0053
G	585.02±10.04	1.7153
H	594.44±2.90	0.4877
I	655.41±16.23	2.4765
J	563.79±14.92	2.6467
K	551.16±11.07	2.0090
L	563.7±16.1170	2.8591

Table 3: Percentage content of the different brands of paracetamol tablets analyzed

Sample Code	Amount Declared (mg)	Amount Found (mg)	% Content	Remarks
A	500	515.65	103.13±2.44	Passed
B	500	632.15	126.43±4.81	Failed
C	500	482.15	96.43±1.98	Passed
D	500	465.15	93.03±1.32	Failed
E	500	492.90	98.58±1.26	Passed
F	500	530.20	106.04±2.71	Failed
G	500	602.30	120.46±11.12	Failed
H	500	528.20	105.64±9.59	Passed
I	500	476.70	95.34±3.54	Passed
J	500	532.95	106.59±7.21	Failed
K	500	540.25	111.01±4.60	Failed
L	500	546.85	109.37±0.81	Failed

Simple and rapid methods for simultaneous analysis of paracetamol contents were reported and both researchers concluded that the two methods can be applied for the analysis of paracetamol content in tablets [9, 10]. But it was further stated that the RP-HPLC method was more reliable [10].

The HPLC method showed good accuracy and reproducibility for determination of paracetamol in paracetamol tablets. The high value of correlation coefficients from the regression equation exhibited good linearity for the methods (Figure 2). Also, the coefficient of variance (% CV) less than 5 for both inter and intraday precision (Table 1). The chromatograms showed high resolution, specificity and reproducible peaks as there were no interference peaks observed as shown in Figure 1a and b. The inter and intraday precision coefficient of variance for 2 and 8 µg/ml were less than 5% and their percentage recovery was more than 90% as shown in Table 3. From the results, it was deduced that about 41.7% of the brands passed while 58.3% failed the specification of the BP for paracetamol content in paracetamol tablet, which should range from 95-105% [11].

According to NAFDAC, fake or counterfeit drug products can be those without active ingredients or with insufficient active ingredients and those with active ingredients different from what is stated on the label claim. Also, included in the definition are clones of fast moving drugs packaged to look like the genuine original brand. Thus, it can be argued that some of the tablets whose content failed to conform to the BP specification might be categorized as fake or counterfeit drug according to any of the aspects of the definition quoted above.

It was reported that 32% out of 89 samples collected from Nigeria and Thai failed in the assay determination due to exposure to high temperature and humidity [12]. Thus, it can be argued that some of the tablet brands that failed to conform to the BP specification are counterfeited drug products [11]. It has been observed that drug counterfeiters have succeeded overtime largely due to lack of awareness and lack of cooperation among stakeholders nationally and internationally. It was also reported in 1998 that a reduction in drug content may be attributed to exposure to sunlight [13].

The HPLC quantitative analysis procedure was fast and accurate for paracetamol analysis and can be used for routine analysis. Furthermore, there is need for constant post-marketing survey by the regulatory bodies and policy makers in the country in order to amend cases of counterfeiting and substandard drugs.

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