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**Short Communication**

# A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY OF ACTIVE COMPONENT IN PARACETAMOL SYRUP CONTAMINATED BY *PENICILLIUM EXPANSUM.*

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## ABSTRACT

In this study, a high performance liquid chromatographic assay was carried out at room temperature to determine the concentration of active ingredient in paracetamol syrup contaminated by *Penicillium expansum.* The drug samples were extracted in methanol while the mobile phase was methanol: water (20:80 $^{v}/_{v}$) mixture. A volume of 20ul of various concentrations of reference standard (4-aminophenol), contaminated and non-contaminated paracetamol syrups were injected separately in triplicate into a $C_{18}$ analytical column under reverse phase chromatographic conditions. The mean peak height, peak area and retention time for the contaminated sample were 3.47mUA, 23.58mUA$^2$ and 3.51minutes, whereas the non contaminated sample had an average peak height of 368.53mUA, peak area of 2470.04mUA$^2$ and retention time of 3.13 minutes. The standard curve generated from the data yielded perfect linearity with correlation coefficient, r=0.9990. The concentration of paracetamol in the contaminated sample was 0.36ug/ml while the control had 37.53ug/ml. The active component in the contaminated Paracetamol syrup was completely metabolized and transformed into a metabolite with an average peak area of 300.55mUA$^2$, peak height of 20.05mUA and retention time of 3.15 minutes, by *Penicillium expansum.* These results confirm that *Penicillium expansum* can degrade some pharmaceutical drugs and transform them to unrelated forms.

**Keywords:** *Penicillium expansum,* paracetamol syrup, HPLC assay.

## INTRODUCTION

Paracetamol syrup is one the best selling analgesic and anti-pyretic drugs in Nigeria and the world over. It is usually given to infants as first line therapy for feverish conditions. Paracetamol syrup contains acetaminophen or 4-acetylaminophenol as the major active ingredient, and like many other pharmaceutical preparations, it also contains a variety of other excipients in a complex pharmaceutical state which help to make the formulation efficacious, stable and sufficiently elegant to be acceptable to patients. Some of these ingredients are good sources of nutrients, carbon and energy for microbial growth and replication.

Microbial contamination of drugs is a major problem in attaining health care delivery around the world. This is so because contamination often results in a loss of potency and spoilage of the products. Administration of such spoiled syrups on infants may pose a serious health risk (Brooks and Antai, 2005). Biodeterioration of paracetamol syrups may occur due to the microbial degradation of the active ingredients of drugs, resulting in biomass production, diminished potency and loss of one or more performance characteristics; hence the drug becomes therapeutically ineffective. The degradation products may also cause adverse reactions in patients. Therefore, it is justifiable to determine the amount of active ingredient in the contaminated syrup in order to ascertain the effect of this contaminating mold on the overall bioconversion of this pharmaceutical drug.

For the assay of paracetamol, different methods have been reported including spectrophotometry (Marti *et al.,2000*) and sequential injection analysis (Dalibor *et al.,* 2004). However, considering the properties if the paracetamol syrups investigated in this work such as mid-polarity, thermolability and low volatility, the use of HPLC method was quite adequate (United State Pharmacopoeia, 2002). High performance liquid chromatography represents advanced analytical method for separation and simultaneous determination of substances in mixtures. The method has their advantage of limited sample consumption, short analytical time, high resolution and ability to degradative products.

The present study was carried out to determine the concentration of active ingredient in paracetamol syrup

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contaminated by *Penicillium expansum.* This was with the view to evaluating the effect of fungal contamination on the active component of the syrup.

## MATERIALS AND METHODS

Sources and collection of samples

The paracetamol reference standard (4-aminophenol) and methanol were the sigma brand, obtained from the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. The expired and unexpired samples of paracetamol syrups were Emzor brand, purchased locally from pharmacy shops and patent medicine stores in Calabar, Southern Nigeria. Oxoid brand of Sabouraud dextrose agar was used for the isolation of the fungal contaminant from the samples. All other chemicals used were of analytical grade quality.

Sources and collection of samples

One milliliter of each expired and unexpired samples of paracetamol syrup were aseptically taken and homogenized separately with 9.0ml of sterile peptone water. Serial dilutions up to $10^{-5}$ were carried out using this diluent and 0.01ml of the last two dilutions were inoculated on Sabouraud dextrose agar using pour plate technique. The plates were incubated at room temperature for up to 5 days.

Isolation and identification of the fungi isolate

The *Penicillium expansum* was isolated from expired and unexpired paracetamol syrups obtained from patent medicine stores.

Pure culture was made on fresh SDA by repeated subculture. Purified colonies were stored in slants of the same media at $4^{o}$C and thereafter characterized and identified using the standard taxonomic scheme of Barnett and Hunter (1972).

Processing of samples for chromatographic analysis

Mobile phase

The optimal mobile phase for separation of paracetamol was methanol:water (20:80 $^{v}/_{v}$). The mobile phase was degassed before used by means of automated degasser unit containing helium in the HPLC system.

Reference standard solution

A 1000ug/ml of paracetamol reference standard (4-aminophenol) was prepared in 10% ($^{v}/_{v}$) methanol to produce reference standard stock solution. This stock solution was diluted serially with HPLC water to give various concentrations of working standards, ranging from 10-100ug/ml, which were used for the preparation of standard curves.

Sample preparation

The paracetamol syrups used for the analysis had a declared content of 120mg/5ml. A 40ug/ml of the contaminated and non-contaminated (control) samples were prepared by diluting 167ul each sample separately in 10l of methanol. This volume was transferred to 100ml calibrated flask, made up to the 100ml mark HPLC water, and mixed by ultrasonication for 10min. A quantity of this preparation was filtered into 1.5ml capped polyethylene microcentrifuge tubes and the content in each was vortex-mixed at high speed for 2min.

Apparatus

The HPLC system use in this study was the Agilent 1100 series and comprised of water auto sampler syringe system, compression module, silica steel column (Coulter, USA) measuring 46mm x 25cm with C–18, 5um ultrasphere ODS cartridge insert, and variable UV detector. Flow connection lines were aspirated through the selection valve and then delivered to the column and to the detector. The HPLC system was interfaced with HP56 laser jet 110 computer systems and HP 120 laser jet printer for data processing and printing.

Chromatographic assay

All chromatographic separations were carried out at room temperature. A volume of 20ul of the different concentrations of the reference standard was injected into the HPLC column using a 20ul – syringe. The flow rate was set at 1.0ml per minute and the UV detection wavelength at 254nm. Eluting peaks were plotted and quantified by the PC system. Average areas were calculated and used for the preparation of a standard curve. The method was validated at three concentration levels for intra- and inter- day repeatability of the retention times.

Test and control samples were similarly analyzed. Identification of peaks in the paracetamol syrups was based on comparison of the retention time of the compound in the standard solution. All the samples were injected in triplicate and the mean values of peak height, peak area and retention time were used for data acquisition.

## RESULTS

The chromatograms of HPLC analysis of contaminated and non-contaminated paracetamol syrups, as well as the reference standard are presented in figure 1. There were reductions in the concentration of active ingredient in the contaminated samples when compared with the control (PSO). The average concentration of active ingredient in samples contaminated by *Penicillium expansum* ($PS_2$) *was* 0.36ug/ml while the non-contaminated sample (control), had 37.53ug/ml of active ingredient There was a complete conversion of the active ingredient in the sample

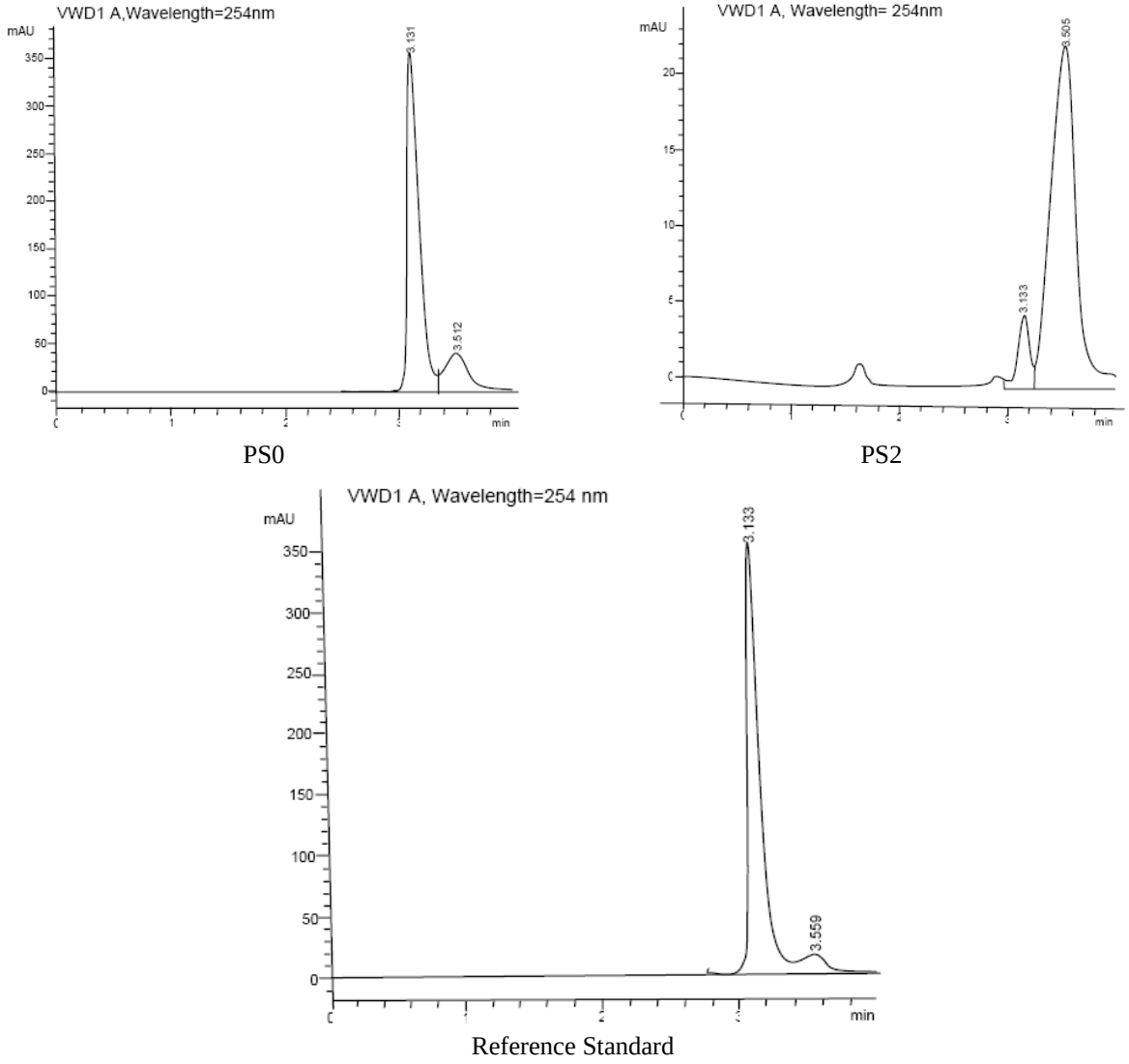


Fig. 1. Chromatograms of contaminated and non-contaminated paracetamol syrups
PS0 = Control (non-contaminated) , PS2 = Contaminated Paracetamol syrup with *P. expansum*

contaminated with *P. expansum* ($PS_2$) to an unrelated metabolite.

The chromatograms showed differences in peak heights and peak areas of contaminated and non-contaminated paracetamol syrups. Specifically, the average peak height and peak area of $PS_2$ reduced drastically to 3.47mAU and 23.58mAU$^2$ respectively. The average peak height, peak area and retention time in the control samples were 368.53 mAU, 2470.04 mAU$^2$ and 3.13 min. respectively, whereas the average retention time in the contaminated sample was 3.51min.

## DISCUSSION

In this study, the effect of fungal contamination on the content and concentration of active ingredient in paracetamol syrup was assessed. There was a reduction in the concentration of the active component in the contaminated sample when compared with the control. The reduction could be as a result of biodegradation of the active ingredient. The level of reduction of active ingredient in the samples also varied, probably due to differences in the resource requirement for fungal growth and metabolism.

A 99.04% reduction in the concentration of active ingredient was recorded in the paracetamol syrup contaminated by *Penicillium expansum*, indicating that a greater proportion of the active component had been utilized by this mold. Hugo and Russel (1992) reported a 20% reduction of the active ingredient in atropine containing eye drops contaminated with *Pseudomonas* and *Corynebaterium* species. Baird (1981) also reported

that *Penicillium species* were able to utilize substituted acetanilides such as paracetamol solution as a sole source of carbon.

In this study, the HPLC analysis of paracetamol syrup contaminated by *Penicillium expansum* showed that the active component was transformed into a metabolite with average peak area of 23.58mAU$^2$ and peak height of 3.47mAU. The low elution peaks might imply that the metabolite was less active than the original paracetamol. The reference standard and the control (non-contaminated) samples eluted at approximately 3.13mins. after injection into the HPLC system, while the sample contaminated with this mold eluted at a much longer time of 3.15mins. The longer analytical run time might be attributed to the degradation product of the syrup and hence would be expected to elute at a later time. The reaction intermediate produced by this mold gave chromatographically discernable peaks which were quite different from those of the standard and non-contaminated samples. It could be argued that the metabolite did not share similar pharmacokinetic properties with the active paracetamol itself, hence it exhibited a different stereosolectivity. It is also possible that the metabolite might have lost the therapeutic properties of the parent paracetamol due to rapid utilization of the active ingredient by the contamination This corroborates the earlier observation of Baird (1981) that *Pencillium spp.* were able to utilize the active ingredient of paracetamol as carbon source.

The presence of *Pencillium expansum* in paediatric oral paracetamol syrup is of public health importance, because such contaminated product is capable of causing medical complications when administered on paediatric patients. This is a source of concern and calls for strict adoption of good manufacturing practice at all stages of production of this syrup.

It has been reported by Cheesbrough (2000), that some species of *Pencillium* are opportunistic pathogens, causing systematic penicilliosis in Thailand, Southern China, Hong Kong, Vietnam and other parts of Asia. Therefore the presence of this pathogen in paracetamol syrups made in Nigeria may illicit more catastrophic effect in an already debilitating patient who may be receiving several doses of this drug to relieve pains and feverish condition.

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