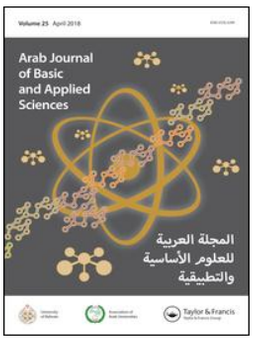

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ORIGINAL ARTICLE

A new method for the microdetermination of *Para*-aminophenol in generic brands of paracetamol tablets

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ABSTRACT

In Nigeria, paracetamol is readily available in several retail outlets where the conditions of storage can be poor leading to elevated levels of *para*-aminophenol (PAP), which is known to be nephrotoxic and hepatotoxic. However, the routine analysis of PAP is mostly by chromatographic separation which requires expensive instrumentation not often available in developing countries. The objective of this research was to develop a sensitive colorimetric method for the quantification of PAP in paracetamol.

The method was based on the diazo coupling reaction between diazotised PAP and chromotropic acid. Various reaction parameters critical for optimal detector response were optimized. The validation of the new method was done following the determination of parameters including repeatability, reproducibility and selectivity using current ICH guidelines. The new method was also applied to the assay of PAP in 14 paracetamol tablet samples.

The calibration was linear between 0.0297 and 0.2229 µg/mL at 470 nm with limits of detection and quantification of 0.0061 and 0.0185 µg/mL, respectively. The recovery was in the range of 95.96 and 102.21 while intra- and inter-day precisions at three different concentrations did not exceed 4.03%. The new method was successfully applied to quantify PAP in paracetamol with percent content varying from 0.14 to 0.21%w/w.

A simple and reliable method for the quantification of PAP has been developed and successfully employed to report, for the first time, the presence of the degradation product at levels beyond the allowable limits in paracetamol dosage forms in Nigeria.

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Paracetamol; para-Aminophenol; Chromotropic acid; Colorimetric microanalysis

1. Introduction

Paracetamol (acetaminophen) is a commonly used analgesic in single and combination preparations that are formulated in a wide range of dosage forms including tablets, syrups, soluble powder, suppositories and injectables. Paracetamol has substantial antipyretic activity and is often included in both over-the-counter and prescription drug therapies for common ailments like malaria. It is therefore a widely sought-after medication in Nigeria that is readily available not only in pharmacies but also in patent medicine drug stores and supermarkets where the handling and conditions of storage can be far from ideal (Orisakwe, Orish, & Aka, 1994). Unwholesome storage of the drug may result in *para* aminophenol (PAP), which is the main degradation product of paracetamol as well as the main impurity from its synthesis, being present at levels in excess of the allowed limits of 0.005% and 0.1% by weight in bulk powder and dosage forms, respectively (BP, 2013). While the presence of PAP in samples of paracetamol might not only be the cause of therapeutic failure but also raises safety concerns as PAP has been shown to be significantly more potent than paracetamol as a nephrotoxicant in animal models (Newton, Kuo, Gemborys, Mudge, & Hook, 1982), so the toxicity potential of the degradant following prolonged consumption as is the practice in most third world settings portends danger over time. It also has hepatotoxic (Fu, Chen, Ray, Nagasawa, & Williams, 2004) and teratogenic activities (Bishop, 2003).

Different analytical methods for the detection and quantification of PAP in paracetamol formulations are described in the literature. These methods range from spectroscopic techniques including second derivative ultraviolet spectroscopy (Yesilada, Erdogan, & Ertan, 1991), colorimetry (Korany, Heber, & Schnekenburger, 1982; Mohamed, AbdAllah, & Shammat, 1997), fluorimetry (Dejaegher, Bloomfield, Smeyers-Verbeke, & Vander Heyden, 2008) to chromatographic techniques such as micellar electrokinetic chromatography (Németh, Jankovics, Németh-Palotás, & Kőszegi-Szalai, 2008) and several liquid chromatography techniques. The latter

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technique has also been variously adapted with regard to detection which includes amperometric (Wyszecka-Kaszuba, Waronwna-Grzeskiewicz, & Fijalek, 2001), voltametric (Liu, Li, Dong, & Wang, 1996), and spectrophotometric (Nageswara and Narasaraju, 2006). A flow injection method with UV detection (Bloomfield, 2002) and voltametric methods which fundamentally involve electrochemical oxidation of the amine to quinone imine are also described in the literature (Safavi, Maleki, & Moradlou, 2008; Wyszecka-Kaszuba et al., 2001). However, the use of some of these methods requires expensive equipment and reagents not readily accessible in developing countries as well as extensive sample preparation procedures. The objective of this study was therefore to develop a simple, cost-effective colorimetric method that is sufficiently sensitive and accurate to serve as an alternative to official methods in the routine detection and quantification of PAP in generic brands of paracetamol available in the Nigerian market using chromotropic acid. Chromotropic acid has found applications in the determination of formaldehyde (Shariati-Rad, Irandoust, & Mozaffarinia, 2016; Gasparini, Weinert, Lima, Pezza, & Pezza, 2008), methanol (Gazani, Shariati, & Rafizadeh, 2017), nitrate (West and Ramachandran, 1966) and dipyrone (Sakiara, Pezza, Melios, Pezza, & de Moraes, 1999). Chromotropic azo derivative has also found usefulness in the determination of trace oxalic acid (Zhai, Zhang, & Liu, 2007). Very recently, chromotropic acid was used for the sensitive determination of gabapentin in dosage forms (Adegbolagun, Thomas, Aiyenale, & Adegoke, 2018).

2. Materials and methods

2.1. Materials and reagents

Fourteen generic paracetamol tablets belonging to six different brands were used for the study. Each brand was sourced from at least two of the following: open market, patent medicine stores and pharmaceutical shops in Ibadan, Nigeria.

All reagents used were of analytical grade while solvents were used without further purification. These include chromotropic acid (Sigma Aldrich USA), *para* aminophenol (BDH UK), sodium hydroxide (Qualikem India), hydrochloric acid (BDH UK), sodium nitrite (Qualikem India). Distilled water was used for all preparations and dilutions. A 10% w/v aqueous solution of sodium nitrite as well as equimolar concentrations of *para* aminophenol and chromotropic acid solutions were prepared in double distilled water.

2.2. Instrumentation

Mettler Analytical balance (Ohaus USA), Visible spectrophotometer 6405 (Jenway, UK), Thermostated water bath (Langford UK).

2.3. Analytical procedure

A 0.3 mL aliquot of the PAP solution (0.003122 M) was transferred into a beaker maintained in an ice-bath with a mechanism for continuous stirring. Aliquots of an equal volume of sodium nitrite solution and 0.12 mL of 2 M HCl solution were added successively to the beaker and the reaction allowed to proceed for 20 min with stirring. Thereafter, 13 mL ice-cold distilled water was added to quench the reaction. Freshly prepared diazonium solutions were used for subsequent work.

2.4. Evidence of coupling reaction

Samples for spot test and thin layer chromatography analysis were prepared by mixing 0.5 mL of the diazonium with 0.5 mL of the chromotropic acid (0.003122 M) solution. The colour change was noted immediately and after 20 min following incubation at room temperature and at 70 ^{0}C. The TLC examination of the adduct formed, the diazonium and chromotropic acid solution, was carried out using a mobile phase consisting of ethyl acetate: methanol: water (6:3.5:0.5).

2.5. Selection of analytical wavelength

A 0.5 mL aliquot of the diazonium was added to an equal volume of the coupling agent in a test tube and then incubated at room temperature for 10 min, after which the reaction was stopped by cooling in an ice bath and the volume made up to 5 mL with methanol. The UV-visible spectrum between 190 and 800 nm of the solution was acquired with methanol as blank; the spectra of 0.5 mL aliquots of the diazonium and chromotropic acid solutions made up to 5 mL with methanol were also separately acquired.

2.6. Optimisation of reaction variables

2.6.1. Optimisation of the coupling temperature

The optimization of coupling temperature was achieved using the method of steepest ascent at temperatures of 30, 50, 60 and 70^{0}C following incubation for 5- and 20-min periods at each temperature level. Separate mixtures of 0.5 mL aliquots of the diazonium and chromotropic acid solutions in test tubes were incubated as described, after which the reaction was stopped and the volume made up to 5 mL with methanol. The absorbance of each solution at 470 nm was determined using methanol as blank.

2.6.2. Optimisation of coupling time

The optimal reaction time for diazo coupling was determined following incubation of separate mixtures of 0.5 mL aliquots of diazonium and chromotropic acid solutions at the selected temperature of 60^0C for varying time intervals which included 0, 2, 5, 10, 15, 20, 25 and 30 min. In each instance, the reaction was stopped by cooling the reaction mixture in an ice bath and then diluting the volume to 5 mL with methanol. The absorbance of each solution was then determined at 470 nm using methanol as the blank solvent. Each determination was done in duplicate.

2.6.3. Optimisation of dilution solvent

The effect of solvent used to terminate the reaction following incubation at optimal temperature and time was investigated using water, methanol and ethanol.

2.7. Determination of stoichiometric ratio

Job's method of continuous variation was employed to determine the stoichiometric ratio for maximum adduct generation (Rose, 1964). Increasing volumes of the diazonium solution (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0) were transferred separately into different test tubes. The volumes were then made up to 1 mL with chromotropic acid solution after which the reaction mixture was maintained at 60 for 10 min. The reaction was thereafter stopped by cooling in an ice bath and dilution of its volume to 5 mL with methanol. The absorbance was determined at 470 nm using methanol as the blank solvent. Each determination was carried out in duplicate.

2.8. Validation

The calibration curve was generated from the three-day average of curves using 0, 0.02, 0.03, 0.04, 0.045, 0.05, 0.1 and 0.15 mL (equivalent to 0.0297, 0.0446, 0.0597, 0.0669, 0.07434, 0.1487, 0.2229 g/mL) of diazotised PAP. To each of the volumes in separate test tubes, 0.5 mL of chromotropic acid solution was added, vortex mixed for 10 s and then incubated at 60^0C for 10 min. Thereafter the reaction was stopped by cooling and each reaction mixture made up to 5 mL with the required volumes of methanol. Each determination was carried out in triplicate. The accuracy and repeatability of the new method was determined on three successive days at three concentrations of the analyte as stipulated by the International Conference on Harmonisation (ICH, 2011). The precision of the method was estimated as percent relative standard deviation while the limit of detection (LOD) and limit of quantification (LOQ) were determined according to current ICH guidelines as the ratio of the 3.3 and 10 of the standard deviation of the blank signal (n = 6), respectively, divided by the slope of the calibration curve.

2.9. Interference studies

A 5 mg quantity of each of a selected list of common excipients (talc, magnesium stearate, starch, lactose) and a mixture of all of them was added to separate aliquots of 0.045 mL of diazotised PAP (equivalent to 0.0669 μg/mL) in test tubes and then coupled with 0.5 mL chromotropic acid solution. The reaction mixture was then incubated at 60^0Cfor 10 min after which it was cooled in an ice bath and the volume made up to 5 mL with methanol. The absorbance of each solution was determined at 470 nm using methanol as blank. Each determination was done in quadruplicate.

2.10. Analytical signal stability

Samples of the adduct solutions were split into either of two groups: wrapped with aluminium foil or exposed to sunlight. The absorbance values of the solutions at 470 nm were determined at half-hour intervals for a total period of 180 min.

2.11. Dosage form analysis

2.11.1. Assay of active pharmaceutical ingredient

The 14 samples were analysed for their active ingredient content using the official British Pharmacopoeia Commission 2013 method.

2.11.2. Analysis of PAP in commercial paracetamol dosage forms

Four tablets from each of the samples were powdered and transferred to a 10 mL volumetric flask to which about 6 mL of distilled water was added and then shaken thoroughly for 20 min. The mixture was made up to 10 mL with water and then filtered. A 0.3 mL portion of the filtrate was used as stock solution for the diazotization, as previously described. A 0.045 mL aliquot of the diazonium ion of PAP produced was transferred into six test tubes for each brand. A 0.5 mL aliquot of chromotropic acid solution was added to each of the test tubes and the reaction allowed to proceed at 60^0C for 10 min. Thereafter the reaction was stopped by cooling in an ice bath and making up the volume to 5 mL with methanol. The absorbance of the reaction mixture was taken at 470 nm with methanol as blank.

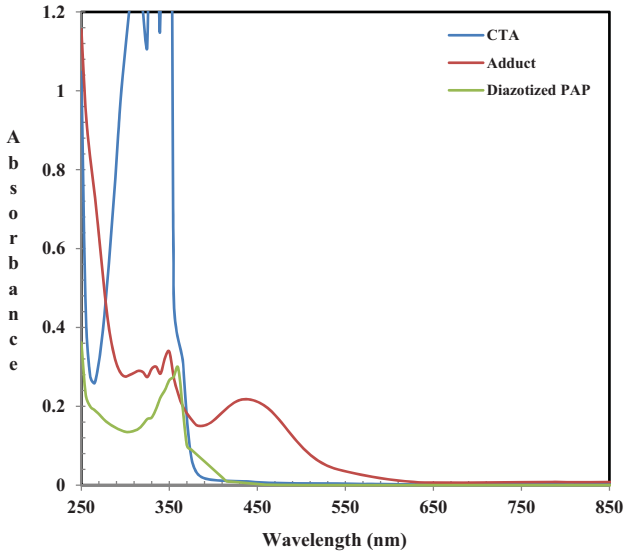


Figure 1. Overlaid spectra of diazotised PAP, chromotropic acid (CTA) and the adduct formed.

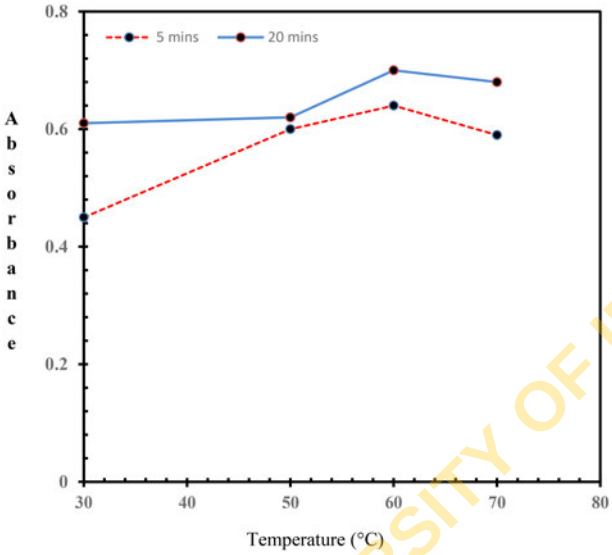


Figure 2. Optimization of coupling temperature.

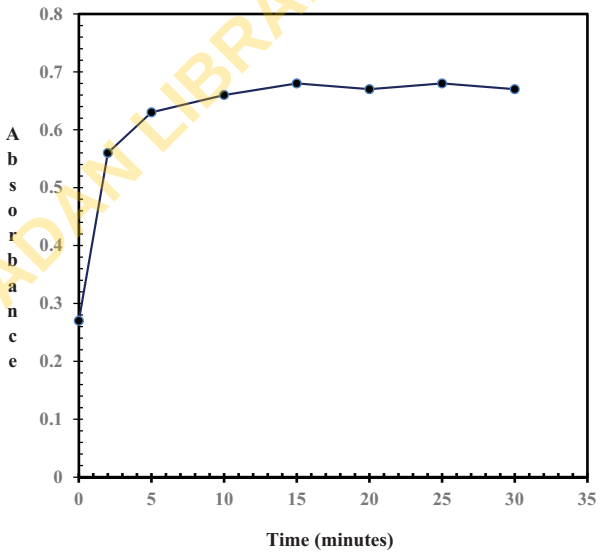


Figure 3. Optimization of coupling time at 60^{0}C.

2.11.3. Recovery studies of spiked PAP into paracetamol tablets

The standard addition method was used to investigate matrix effects on the recovery of the new method. Five brands of the generic paracetamol tablets were used for the assessment. Four tablets of a brand of paracetamol were powdered and transferred to a 10 mL volumetric flask which was then made up to volume with double distilled water. After equilibration for 20 min, the mixture was filtered to obtain a clear filtrate. Increasing amounts (0, 10, 20, 30 40 µL) of a standard solution of PAP were added to equal aliquots of the filtrate contained in various test tubes. Each mixture was diazotised and employed in coupling with chromotropic acid solution under the established optimal conditions before determination of the absorbance reading at 470 nm. The procedure was repeated for the other brands of paracetamol.

3. Results

3.1. Evidence of coupling reaction

The spot test revealed the instantaneous formation of an orange adduct at room temperature with deeper intensity at 70^{0}C. TLC examination also confirmed the formation of a new adduct as the R_f values of the product (0.853) were distinct from those of either the diazonium (0.441) or coupling agent (0.588).

3.2. Selection of analytical wavelength

The analytical wavelength was selected by the inspection of the overlaid spectra of the diazotised PAP, chromotropic acid and adduct as shown in Figure 1. There is a bathochromic and hyperchromic shift in the spectrum of the adduct with a new peak appearing at around 450 nm. However, optimal differences in absorptivity between the azo adduct and

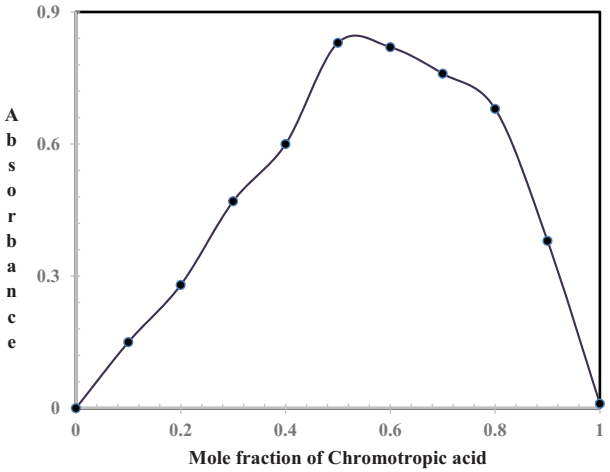


Figure 4. Variation of absorbance with mole fraction of coupling agent.

chromotropic acid were obtained at 470 nm. This wavelength was therefore selected as analytical wavelength as it is devoid of interference from other absorbing species.

3.3. Optimisation of reaction variables

The optimal coupling temperature and time were established at 60^{0}C as shown in Figure 2 and 10 min as shown in Figure 3, respectively. The decline in absorptivity at higher temperatures is probably due to the breakdown of the azo linkage. In order to select the most appropriate dilution solvents the use of water, methanol and ethanol were separately investigated. Methanol gave higher absorbance values with better regression equations and coefficient of determination. This might not be unconnected with the ability of methanol to provide an excellent medium in which the azo adduct is maximally stable without any possibility of azo dye decomposition as may occur if water is used.

3.4. Stoichiometry and reaction mechanism

The plot of the absorbance value of the adduct as a function of the mole fraction of the coupling agent reveals a stoichiometric ratio of 1:1 as shown in Figure 4.

The new method is based on the electrophilic attack of the diazonium on the activated ring system of chromotropic acid. A stoichiometric ratio of 1:1 gave the maximum signal response which is indicative of the formation of a mono azo dye following coupling. This is consistent with the proposed chemical structure of the azo adduct formed as reported in a number of method development studies involving the use of chromotropic acid as the coupling agent for diazotised pharmaceutical agents (Abou-Attia, Issa, El-Reis, Aly, & El-Moety, 2002; Revanasiddappa and Veena, 2007; Darweesh, Al-Haidari, Mohammed, & Dikran, 2017). However, we disagree with the conclusion in these reports that the azo linkage is *ortho* to the hydroxyl group because in that case a 1:1 adduct is unlikely as two of such *ortho* positions exist in the coupling agent with both being chemically equivalent with little or no possibility of steric interference from each other. We therefore propose that the azo linkage is *para* to the activating hydroxyl group as shown in Scheme 1. It is well documented that hydroxyl group on naphthyl rings are *ortho* and *para* directing when present at position 1 and 2, respectively, and that substitution in between groups that are *meta* to each other is less probable due to steric effects (Morrison and Boyd, 1992). Thus, the *para* position to the hydroxyl is more accessible to the incoming electrophile, which on forming the azo linkage sterically hinders the approach of a second diazonium molecule towards the *para* position of the *peri* (second) hydroxyl group. This is in agreement with the observation that a 1:1 adduct is almost always formed between chromotropic acid and diazonium ions.

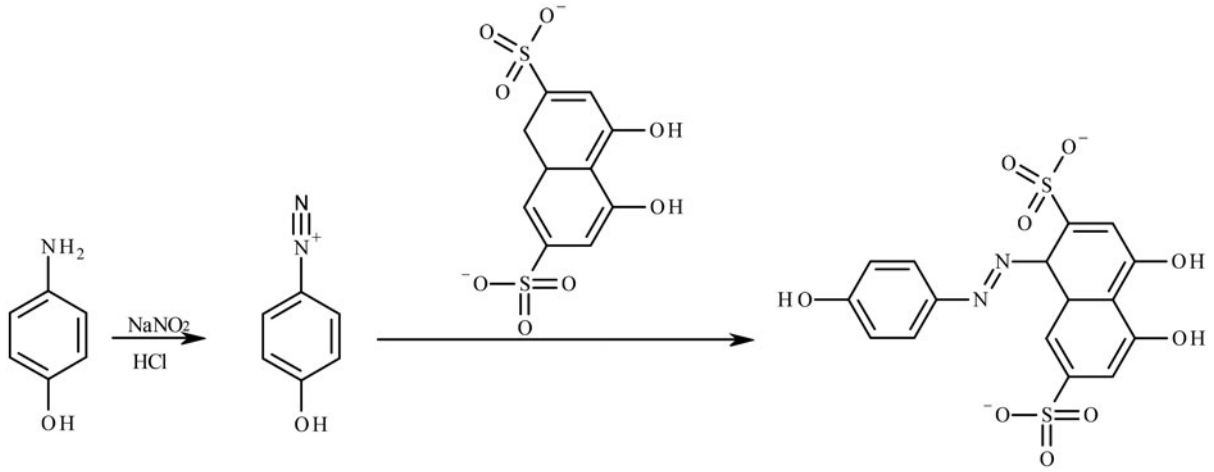
In addition to the foregoing, the optimized 3 D structures of the likely *ortho* and *para* products are presented in Figure 5. The structures present some implications for stability of the azo adducts formed. For the *para* azo adduct, on the naphthalene residue, the sulphonic acid moiety which exists as the fully negatively charged radical is in close proximity to the next proton. This will dramatically account for stability of the molecule because possibilities of hydrogen bonding and other ion-pair interactions are promoted thereby. However, for the *ortho* azo adduct (Fig. 5a), the crowding is clearly evident which will obviously lead to instability. The formation of the *para* azo adduct which allows for proper and adequate staggering is highly favoured.

3.5. Validation studies

A linear correlation was obtained at wavelength of 470 nm and at concentrations of 0.0297–0.2229 μg/mL of *para*-aminophenol. The analytical and validation parameters for the new method are also presented in Table 1.

The results for the assessment of accuracy and precision are presented in Table 2. As shown in Table 2, the percentage relative error for intra-day accuracy did not exceed 4.03%, with recovery of 95.96–102.21%, indicating good accuracy. The percentage relative standard deviation for the intra-day precision did not exceed 3.6%, indicating good repeatability. For inter-day accuracy, the percentage relative error did not exceed 4.03%, with recovery of 96.0–101.47%. The percentage relative standard deviation was 0.25–3.30%, indicating good reproducibility.

While spectrophotometric detection with chromatographic separation has been frequently used for the quantification of PAP in paracetamol, only a few



Scheme 1. Proposed mechanism of reaction for the formation of azo product.

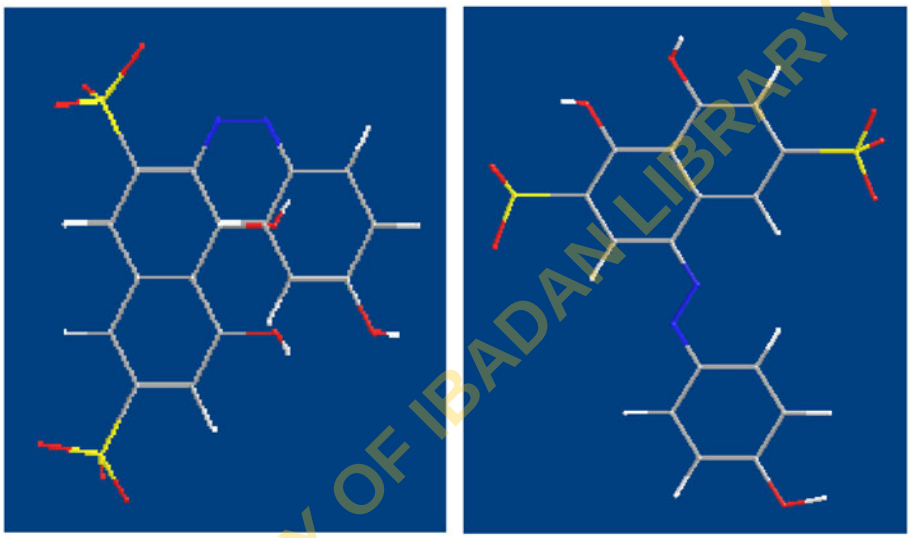


Figure 5. 3D optimized structures for the *ortho* (a) and *para* (b) adducts of the product between CTA and PAP.

Table 1. Analytical and validation parameters for the proposed method.

Performance parameter	Value
Beer's law limit (μg/mL)	0.0297–0.2229
Limit of detection (μg/mL)	0.0061
Limit of quantification (μg/mL)	0.0185
Molar absorptivity (L mol^{-1} cm^{-1})	3.028×10^4
Slope ± SD	2.1977 ± 0.124
Intercept ± SD	0.0298 ± 0.011
Coefficient of determination	0.998

colorimetric methods are available as stand-alone procedures in the literature. These include those based on the oxidation of PAP with 3-cyano-N-methoxypyridinium perchlorate (Korany et al., 1982) and either of Ce^{4+} or Fe^{3+} (Mohamed, AbdAllah, & Shammat, 1997) which were determined at 10–60 and 1–10 μg/mL, respectively. Our method, however, offers a number of advantages: the improved sensitivity and lower Beer's concentration range permit the micro analysis of PAP at levels not possible with these two methods as well as better recoveries. Our method is also simple, safe, employs readily available laboratory reagents and does not require additional synthesis of reagents/intermediates as is the case with the use of the pyridine derivative which is known to be nephrotoxic, hepatotoxic and reduces male fertility (NIOSH, 2018).

3.6. Interference studies

Method selectivity studies revealed a recovery range of 95.40–102.09% indicating that there is no interference with the different excipients except gelatin which showed high absorbance and recovery values. This is probably due to the acid and/or thermal catalysed degradation of the polypeptide into amino acids which causes dispersion in the reaction mixture.

3.7. Analytical signal stability

There was no significant difference between the mean absorbance of the two groups of wrapped and exposed samples to diffuse light (*t*-value =

Table 2. Accuracy and precision of new method.

	Intra-day*			Inter-day**		
Amount added (μg/mL)	Mean recovery (%)	RSD (%)	Relative error (%)	Mean recovery (%)	RSD (%)	Relative error (%)
0.0297	95.96	0.13	4.03	96.0	0.25	4.03
0.0743	100.65	3.6	0.66	101.47	3.30	0.11
0.1487	102.21	1.8	2.21	100.60	1.70	0.60

*$n = 4$ for each concentration level,
**$n = 12$ for each concentration level.

Table 3. Percent content of PAP and PCM in generic paracetamol tablets.

Brand	City/Country of manufacture	Vendor	Batch number	Expiry date	Percent PCM found	Percent PAP found
A	Lagos, Nigeria	*	1140V	03/21	100.4	0.21
		+	5883U	12/20	95.9	0.17
		#	1896U	05/20	98.7	0.14
B	Lagos, Nigeria	*	185W	11/18	99.8	0.17
		+	012X	01/19	102.7	0.16
		#	031W	05/18	100.0	0.15
C	Agbara, Nigeria	+	A160098	12/20	100.8	0.20
		#	A151794	10/20	100.7	0.15
D	Ibadan, Nigeria	*	A6038A	01/19	103.5	0.21
		+	A6063A	01/19	103.6	0.20
E	Oyo, Nigeria	*	15133	11/18	103.4	0.20
		+	15145	11/18	104.4	0.16
F	Osun, Nigeria	*	A0188	12/17	103.9	0.17
		+	A0175	12/17	104.6	0.16

*open market/street vendor;
+patent medicine stores;
#registered Pharmacy outlets.

Table 4. Standard addition method for the assay of spiked PAP in paracetamol tablets.

Brand	Vendor	% Content of PAP ± SD	nMatrix effect (%)
A	#	0.138 ± 0.083	98.35
B	*	0.165 ± 0.042	97.06
C	#	0.155 ± 0.019	103.33
D	+	0.204 ± 0.029	102.01
E	+	0.161 ± 0.038	100.63

*open market/street vendor;
+patent medicine stores;
#registered Pharmacy outlets;
n=ratio of recovery from standard addition to three-day calibration curve.

0.025) as the azo product was stable over the 180-min period. The profound stability of the azo adduct produced between diazotised PAP and chromotropic acid accounts for this lack of difference in the absorptivities whether the samples of the azo adducts are wrapped away from or exposed to diffuse light. This will allow for analysts to work on the samples without undue decomposition in cases where large amounts of samples are to be handled.

3.8. Dosage form analysis

3.8.1. Determination of content of active ingredient in the generic brands

The percent contents of the 14 samples of paracetamol tablets were determined using the official method. All samples passed the weight uniformity test with the label claims of the active content also within the specified limits of 95–105% w/w as shown in Table 3.

3.8.2. Analysis of PAP in commercial paracetamol dosage forms

The extreme solubility of PAP in water was exploited in selectively extracting it from the tablet dosage form for subsequent diazotisation and analysis. The extracts obtained from all the brands were clear, colourless solutions which turned yellow upon diazotisation. PAP was detectable in all the samples at levels expressed in percent by weight of the active pharmaceutical ingredient, as depicted in Table 3.

3.8.3. Analysis of PAP spiked into paracetamol dosage form

The matrix effect was evaluated on five brands of paracetamol with the amount of PAP in each determined from the ratio of the Y-intercept to slope of the standard addition calibration curve. The recovery of PAP as well as the matrix effect (calculated as the ratio of the concentration of PAP obtained with standard addition to that obtained with three-day calibration curve) in five brands of paracetamol are presented in Table 4. In the absence of the adoption of an official method, this proof of accuracy from the spiking experiments justifies the suitability of the new method for the analysis of PAP in paracetamol generics. As evident from the results, the amount of PAP recovered still falls within the accuracy obtained when PAP was analysed directly. The results showed that the sensitivity of the new method is not compromised by matrix influence. This proves the suitability and reliability of the new method to determine PAP in decomposed samples of paracetamol tablets.

This report represents the first investigation of its kind in the quality control of impurity in generic paracetamol tablets in the Nigerian market. Although all the samples passed the active ingredients content assay, PAP was present in amounts exceeding the allowable limits in all the 14 samples. It thus appears that the greatest concern with generic brands of paracetamol in Nigeria is not therapeutic failure but a public health one as PAP is documented to possess not only hepatotoxic, teratogenic activities and might indeed be responsible, rather than the N-acetyl-*p*-benzoquinoneimine, for paracetamol-induced methemoglobinemia (McConkey, Grant, & Cribb, 2009), but it is at least five times more potent as a nephrotoxicant than paracetamol in animal models (Newton et al., 1982). Two pathways have been previously proposed for PAP nephrotoxicity which include auto-oxidation to aminophenoxy radical and benzoquinoneimine (Lock, Cross, & Schnellmann, 1993) and secondly, via the formation of a toxic glutathione conjugate (Klos, Koob, Kramer, & Dekant, 1992). In an *in vitro* study on isolated renal slices, PAP within 30 min of incubation at concentrations as low as 0.1 and 0.5 mM depressed gluconeogenesis and all adenine molecules, respectively, prior to Lactate Dehydrogenase (LDH) leakage, an indicator of loss of membrane integrity after only 45 min (Harmon, Terneus, Kiningham, & Valentovic, 2005). Indeed, an exposure time of 15 min to PAP at concentration as low as 0.25 mM was sufficient to induce an increase in LDH at a later time when compared to controls. Total glutathione (GSH) levels were also reportedly diminished within 30 min by 0.25 mM PAP. While the extent of extrapolation of results from animal studies to humans remains debatable, the study shows the potential public heath danger in the consumption of unwholesome paracetamol products even among casual users. The study also demonstrated that pre-treatment with high concentrations (2 mM) of ascorbic acid completely protected against PAP toxicity. This suggests that the co-administration of paracetamol with ascorbic acid, which helps maintain GSH status, may therefore be beneficial in humans.

An obvious trend in the results of our study is that in comparison with registered pharmacies, higher percent content of PAP was found in samples obtained from the open markets, street vendors and patent medicine stores. It can be explained that storage and handling of pharmaceuticals under such conditions are far from the ideal recommendations of USP and B.P. that paracetamol must be preserved in tight, light-resistant containers stored at room temperature and protected from moisture and heat. A percent decrease of up to 11.3 and 31.9% in the shelf lives of commercial paracetamol tablets (arising from hydrolytic degradation to PAP) following an increase in storage temperature from 25 to 37^0 at relative humidity of 75 and 100%, respectively, have been reported (Ahmad and Shaikh, 1993). In addition, while our study did not match the generic brands assayed to rule out the effects of confounding variables such as date of manufacture, local stores mean humidity and temperature etc., a casual observation of the results shows substantial variation in the PAP content of the various brands. In a study to determine the influence of varying temperature and humidity on the stability of commercial generic paracetamol tablets in their original packaging it was found that the compositional variations of the tablet matrices as well as the moisture barrier characteristics of the packaging materials were critical. In particular, PVC provided excellent water permeation control that maintained equilibrium moisture content which discouraged hydrolytic degradation of the API (Ahmad and Shaikh 1993; 2003).

5. Conclusion

A simple, cost-effective and reliable method for the assay of PAP in paracetamol has been developed. This study has also identified potential contributing factors to *para* aminophenol contamination of generic paracetamol tablets in the Nigerian market. In addition, it has documented, for the first time to the best of our knowledge, the presence of the degradation product at levels beyond the allowable limits. There is a need for regulatory agencies to implement control strategies to limit hydrolytic cleavage of paracetamol products in the Nigerian market.

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Disclosure statement

No potential conflict of interest associated with this work was reported by the authors.

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