

Research Article

Development of a Rapid Chemical Identification System (RCIS) for the Detection of Fraudulently Labelled 5-nitroimidazole Products

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Abstract

Purpose: A simple, reliable and rapid chemical identification system (RCIS) consisting of three colour reactions based on the functional groups in the molecule and two TLC methods was developed for preliminary detection of the 5-nitroimidazole drugs.

Methods: Three members of this group of drugs (tablet form) available in the Nigerian market and labelled M_A and M_B for metronidazole, T_A and T_B for tinidazole and S for secnidazole, were used. The extraction of the active ingredient from the solid dosage form was performed using acetone. The reaction of the extracted drug with zinc and 1M hydrochloric acid at 100 °C converted the nitroimidazole group to a characteristic primary aromatic amine. TLC methods A and B were carried out on GF₂₅₄ plates (5cm x 10cm) to further identify the individual members of the group. TLC method A with mobile phase consisting of acetone, ethyl acetate and ammonia (100:5:1) and method B with mobile phase consisting of acetone, chloroform and ammonia (100:15:1) were developed for the identification.

Results: The aromatic character of 5-nitroimidazole was highlighted in nitric acid when combined with sulphuric acid resulting in an orange colour. 40% sodium hydroxide gave the alkali-induced characteristic orange colour of aromatic amino compounds. All the samples of the various brands gave characteristic colours that distinguished the compounds of the 5-nitroimidazole group as primary or secondary nitroimidazole compounds.

Conclusion: Using the developed method, fraudulently labelled product 5-nitroimidazole antiprotozoal and antibacterial agents can now be detected in approx. 40 min with limited reagents and a simple TLC technique. The method is rugged, simple, and should be particularly handy for use in detecting substandard products of the drug in the drug distribution chain where sophisticated equipment are often not available.

Keywords: 5-nitroimidazole; Fraudulently labeled; Chemical identification system; Antiprotozoal; Antibacterial.

Received: 27 May 2009

Revised accepted: 15 February 2010

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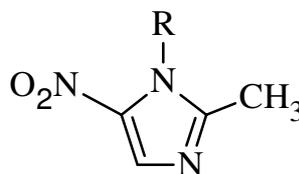
INTRODUCTION

The world is confronted by the reality of large-scale counterfeit production of almost every conceivable drug [1,2]. It is not surprising then that drugs are produced in gross violation of good manufacturing practices (GMP) and in utter disregard of pharmacopoeia standards [2,3]. It is, therefore, desirable that deliberate and fraudulent labeling of drugs with respect to identity should be detected along the distribution chain as part of normal quality assurance processes [4]. There is the need to make available useful methods for accurately validating the authenticity and identity of available medications. This is in view of the fact that the greatest health risk faced by people of the world today is the lack of appropriate medicines for treating both common and life-threatening illnesses [4,5]. The dangers posed by counterfeit products to public health in Nigeria have resulted in control measures by the Agency for Food and Drug Administration and Control (NAFDAC) such as daily enlightenment campaigns in the media [6,7]. A way to complement NAFDAC's efforts against counterfeit drugs is to develop a simple and rapid method of identifying fake products [8]. The growing challenge is halting the distribution and marketing of such medicines at every level of the distribution chain [9, 10].

Equipment with multi-component profiling for the screening of chemicals have been documented in literature but their high cost often render unaffordable to several stakeholder along the distribution chain in several developing countries [11,12]. Furthermore, such equipment requires technical handling and maintenance that are beyond the capacity of those in the distribution chain [13]. A Fast Chemical Identification System (FCIS), as has been used in China since 2005, needs to be developed and compiled for the use of pharmacists and other stakeholders to include drugs such as anti-infective drugs, analgesics, cardiovascular agents, etc. FCIS is usually targeted at the molecular structure

and functional groups in the drug molecule [14-16].

The 5-nitroimidazole compounds are widely used intestinal and extra-intestinal amoebicide. There has been documented evidence of the efficacy of the 5-nitroimidazoles on the causative organism, *Entamoeba histolytica* [17]. The increase in cases of amoebiasis is said to be traceable to counterfeiting [18-20]. The 5-nitroimidazoles most commonly found in the Nigerian market are metronidazole, tinidazole and secnidazole (Fig 1).



R Metronidazole -CH₂-CH₂OH
 2-methyl-5 nitroimidazole-1-ethanol
 Tinidazole -CH₂SO₂C₂H₅
 Ethyl [2-(2-methyl-5-nitro-1-imidazolyl)] ethyl
 sulphone Secnidazole -CH₂-CH(OH)-CH₃
 α, 2-Dimethyl-5-nitro-1H-imidazole-1-ethanol

Fig 1: Chemical structure of 5-nitroimidazole compounds

EXPERIMENTAL

Materials

Branded products of metronidazole, tinidazole and secnidazole in tablet form were purchased from a registered pharmacy in Uyo, Nigeria. Free samples of these drugs were also obtained from May and Baker Plc and Cadila Pharmaceuticals Ltd, both in Lagos, Nigeria. Paracetamol tablets were similarly purchased while pure paracetamol powder was obtained free of charge from May and Baker Plc. Further details are shown in Table 1. Chloroform, acetone, methanol, ethyl acetate, bismuth subnitrate, hydrochloric acid, sodium hydroxide, sulphuric acid, nitric acid, sodium nitrate were

bought from Nanjing Chemical Reagent Company, China, and were of analytical grade. TLC silica GF₂₅₄ plates (5cm x 10cm) were cut from 20cm x 30cm plates (Acres Organics, Belgium).

Preparation of test solution and crystals

Tablets of each of the drugs were crushed in a mortar and reduced into fine powder, and 1 g of the powder was shaken thoroughly in 100 ml of acetone in a conical flask. The drug was extracted into acetone and filtered with a filter paper. The filtrate (50 ml) was allowed to stand at room temperature for 15min to evaporate, yielding crystals. To 50 mg of the crystals of each drug was added zinc dust (50 mg) and 5ml 1M hydrochloric acid in a beaker in a boiling water bath for 5 min. The beaker was removed and then left to stand at room temperature for 20 min. The resulting product was dissolved in 50ml acetone at 50 °C in a water bath and filtered hot. The filtrate was again allowed to stand for 10 min at room temperature to allow the solvent to evaporate, leaving crystals. An amount of the crystals (20 mg) was dissolved in 10 ml chloroform to form the test solution. Similarly, 20mg of paracetamol powder was dissolved in 10ml acetone to form the test solution for paracetamol.

Colour reactions

Nitric acid reaction: To 1ml of the test solution was added three drops of 2M nitric acid. One drop of sodium hydroxide (40 %w/v) was added after 2 min.

Sodium nitrite reaction: 1 g of sodium nitrite and 1 ml of 2M sulphuric acid were mixed in a test tube and cooled in ice. To 1 ml of the test solution was added one drop of the sodium nitrite reagent.

Bismuth subnitrate reaction: Bismuth subnitrate (20 mg) was dissolved in 3 ml of 2M hydrochloric acid. This was diluted with water to 10 ml, 10 g of potassium iodide was added. This reagent (2 ml) was added to 1 ml of the test solution.

TLC identification

Thin layer identification (TLC) identification was carried out on a GF₂₅₄ plate (5cm x 10cm). Mobile phase A consisted of acetone, ethyl acetate and ammonia (100:5:1, v/v) while phase B consisted of acetone, chloroform and ammonia (100:15:1, v/v). Crystals obtained from the similarly treated drug products were dissolved in acetone to give a reference solution of 10 mg/ml. Two

Table 1: Drugs evaluated

Product details	Product code				
	M _A	M _B	T _C	T _D	S
Generic name	Metronidazole	Metronidazole	Tinidazole	Tinidazole	Secnidazole
Brand name & strength	Flagyl 200mg	Emtrin 200mg	Fasigyn 500mg	Amtiba 500mg	Flagentyl 500mg
Manufacturer	May & Baker	Emzor	May & Baker	Cadila	May & Baker
Batch no.	IP 129	XA	C12	BS 104	U132
Manuf./ Expiry date	02-2005/ 01-2010	03-2007/ 03-2010	12-2007/ 12-2011	11-2008/ 10-2011	09-2008/ 09- 2012
Dosage form	Tablet	Tablet	Tablet	Tablet	Tablet

millilitres each of the reference and test solutions were spotted on the plates. The plates were developed, allowed to dry in air for 3 min and placed in an iodine tank for 5 min. TLC was performed in three laboratories in universities in Nigeria to accommodate possible variations in findings arising from differences in environmental conditions. The environmental conditions were 30 °C/45 %RH (University of Uyo); 25 °C/65 %RH (University of Calabar); and 35 °C/27 %RH (University of Sokoto). Three chromatograms were run per brand of drug/sample in each laboratory and the mean R_f taken.

RESULTS

The results of the colour reactions for the various product brands are outlined in Tables 2 - 4 along with R_f values. The colours observed for the generic metronidazole, i.e., M_A and M_B , were similar for each of the reagent/test employed. Nitric acid test was not substituent-specific as orange colour was produced irrespective of the generic type of the nitroimidazole used. Sodium nitrite and bismuth subnitrate distinguished between metronidazole and the other two products as it gave a different colour.

Table 2: Colour reaction tests of 5-nitroimidazole products.

Branded drugs	Nitric acid test	Sodium nitrite test	Bismuth subnitrate test
M_A	Orange	Brown	Brown
M_B	Orange	Brown	Brown
T_C	Orange	Red	Red
T_D	Orange	Red	Red
S	Orange	Red	Red

M, T and S represent metronidazole, tinidazole and secnidazole, respectively, with A and B as brands of metronidazole, C and D brands of tinidazole.

DISCUSSION

Colour reactions

Concentrated nitric acid, when employed with concentrated sulphuric acid, is used to

indicate the aromatic character of a substance. A yellow colour is usually produced when 10% w/v sodium hydroxide is added to a substance with aromatic character, turning orange with excess alkali. The aminoimidazole formed by the zinc/hydrochloric acid reaction during the preparation of the test solution was reconverted via oxidation to nitroimidazole by nitration. Hence, reaction with concentrated nitric acid gave the expected result due to the aromatic nature of 5-nitroimidazoles, as the addition of 40 %w/v sodium hydroxide provided the excess alkali required to produce an orange colour which results from the formation of an aromatic nitro compound.

Table 3: Mean R_f values of various brands of 5-nitroimidazole products evaluated in three laboratories, using mobile phase A (n=3).

Lab,	M_A	M_B	T_C	T_D	S
1	0.460	0.470	0.750	0.730	0.520
2	0.420	0.460	0.750	0.740	0.530
3	0.460	0.470	0.770	0.760	0.540
Mean	0.447	0.467	0.757	0.743	0.530
S.D±	0.023	0.006	0.012	0.015	0.010

M, T, S denote metronidazole, tinidazole and secnidazole, respectively, while 1, 2, 3 denote laboratories in University of Sokoto, University of Uyo and University of Calabar, respectively.

Table 4: Mean R_f values of the various brands of 5-nitroimidazole drugs in three laboratories, using mobile phase B (n=3)

Lab.	M_A	M_B	T_C	T_D	S
1	0.680	0.680	0.540	0.550	0.700
2	0.680	0.640	0.530	0.530	0.710
3	0.680	0.650	0.520	0.530	0.720
Mean	0.680	0.657	0.530	0.537	0.710
S.D±	0.000	0.021	0.010	0.010	0.010

M, T, S denote metronidazole, tinidazole and secnidazole, respectively, while 1, 2, 3 denote laboratories in University of Sokoto, University of Uyo and University of Calabar, respectively.

All the brands of 5-nitroimidazole tested in our experiments gave an orange colour in the nitric acid test (Table 1). A drug that could be fraudulently labelled as an anti-infective is

paracetamol due to its low cost and mild anti-pyretic effect [21]. It has been noted that some labelled anti-infectives only reduce symptoms of illnesses for a while. The colour observed was brown for metronidazole brands, M_A and M_B , in sodium nitrite and bismuth subnitrate tests. Concentrated sulphuric acid reduced the nitro compound to the amino derivative as the sodium nitrite subsequently formed a diazotization derivative with the observed colour based on the extent of conjugation. Sodium nitrite in acidic medium forms diazonium salts with compounds bearing free amino group. The alkyl substituent on the imidazolyl nitrogen distinguishes the 5-nitroimidazole by the characteristic colours observed in sodium nitrate and bismuth subnitrate tests. The alkyl substitution on metronidazole is ethyl alcohol (a primary alcohol) which confers a brown colouration on the compound while tinidazole which bears ethyl sulphone gives a red colouration with the reagents. The N-substitution on secnidazole is a secondary alcohol and this confers a red colour deeper in intensity than observed for tinidazole. The attached substituents on any system containing delocalized electrons either withdraw from the ring or donate electrons to it to a more or lesser extent. The colours, therefore, produced by such compounds depends on the availability and density of available electrons which affect the absorption and transmission of light.

TLC Methods A and B

The mobile phases A and B were designed to make a distinction between the 5-nitroimidazole compounds. In the TLC systems, metronidazole and secnidazole were differentiated from tinidazole by reason of their R_f values. The R_f values of metronidazole brands, M_A and M_B in the various laboratories were similar. A similar finding was observed for the brands of tinidazole, T_A and T_B . The R_f values of the brands of tinidazole were higher than those of metronidazole and secnidazole in the solvent systems (see Table 4). This is probably due to

its ethylsulphone substitution as against the primary and secondary alcohol functional group affiliations of metronidazole and secnidazole, respectively.

The spots produced with solvent system B were more conspicuous on exposure to iodine vapour in the tank. The movement of the solutes was also freer as indicated by R_f values. While the spots for the brands of metronidazole and secnidazole were distinct, the ones for tinidazole exhibited tailing and had a lower R_f (Table 4).

Influence of some variables on the method

The TLC experiments were conducted in laboratories situated in the three major geographic regions of Nigeria spanning 25 – 60 % relative humidity and a temperature range of 25 – 35 °C. These tests were performed in different regions in order to determine if differences in temperature and humidity across the country would influence the tests. Although R_f values were not highly reproducible, the relative pattern of appearance of the spots in the chromatograms was constant and, therefore, useful for drug identification.

Identification system

An identification system for the detection and screening of 5-nitroimidazole preparation was established by combining both the colour reaction and TLC tests. The identification system was rapid (Fig 2) and thus suitable for screening the 5 – nitroimidazole drugs tested in this work. However, since fraudulent labelling and other substandard features (e.g., excess or shortfall in the content of active ingredient in the formulation) often characterise unacceptable products, this identification system does not in any way obviate the need for quantitative evaluation of this group of drugs.

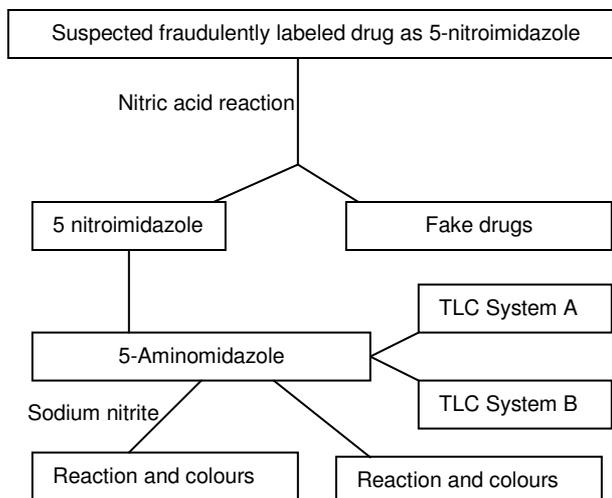


Fig 2: Identification tests for the detection of suspected fraudulently-labeled 5-nitroimidazole drugs

CONCLUSION

The developed identification system based on colours produced by chemical interaction of reagents with the functional groups present in the molecule of 5 – nitroimidazole drugs, and TLC methods can be jointly used to identify fake drugs that are fraudulently labelled as 5-nitroimidazole. This identification system should be useful to those involved in drug distribution, especially community pharmacies and pharmacy departments of hospitals since it will afford them a qualitative assessment of the products. It should, however, be emphasized that the tests evaluated do not eliminate the need for quantitative assessment of this group of drugs as may be necessary.

ACKNOWLEDGMENT

The authors are grateful to JDJIDE Pharmacy, Ikot Ekpene, Nigeria for financial support.

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