Vol. 24 (2021) 131-135

Short Communication

Quality of Metronidazole Benzoate Suspension Products Marketed in Nairobi County, Kenya

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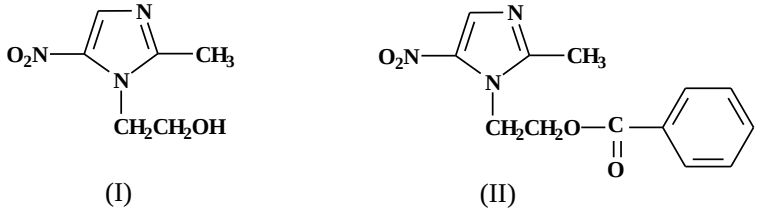
Metronidazole benzoate is an ester derivative of metronidazole utilized in the formulation of oral suspensions owing to its palatability. The drug is liable to hydrolytic degradation during shelf life which could lower assay values and increase free metronidazole content. A medicine quality survey study was carried out in Nairobi County whereby 32 metronidazole benzoate samples representing 13 brands were collected from retail pharmacies. The samples were subjected to British Pharmacopoeia specifications for pH, free metronidazole content and assay. From the results obtained, only nine samples (28.1%) passed all the quality tests performed while five (15.6%), seven (21.9%) and 18 (56.3%) did not comply with pH, metronidazole content and assay specifications respectively. The results obtained demonstrate the existence of substandard metronidazole benzoate products in the market. This underscores the need for regular post market surveillance surveys and execution of appropriate regulatory actions.

Key words: Quality control, assay, metronidazole content, pH, substandard and falsified medicine, degradation

INTRODUCTION

Metronidazole (I) is a 5-nitroimidazole antimicrobial drug that exerts activity against bacterial and protozoal infections. It is clinically applied in the treatment of anaerobic bacterial infections, sexually transmitted infections, amoebiasis, giardiasis and trichomoniasis [1]. A combination of metronidazole, antibiotics and proton pump inhibitors is used for *Helicobacter pyroli* gastritis [2]. Metronidazole kills microbes through the deleterious actions of its nitro-reductive metabolites (nitroso, hydroxylamine and amine) on essential biomolecules including DNA, proteins and membranes [3, 4].

Metronidazole possesses an unpleasant metallic taste that poses compliance and tolerability problems among patients [4]. Consequently, oral dosage forms for adults are coated to mask taste, whilst paediatrics take a more palatable derivative, metronidazole benzoate (II) in suspension form. Metronidazole benzoate is a pro-drug which is hydrolyzed *in vivo* to regenerate metronidazole the active entity. However, the ester can be hydrolyzed as a result of deterioration during shelf life [5].



(I) (II)

Figure 1: Chemical structures of metronidazole (I) and metronidazole benzoate (II)

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Previously, studies in several jurisdictions have demonstrated quality problems with metronidazole benzoate suspensions. A high-performance liquid chromatography (HPLC) analysis of metronidazole tablets and metronidazole benzoate suspensions obtained from retail outlets in Addis Ababa, Ethiopia revealed that all five suspension samples complied with Indian Pharmacopeia specifications for assay [6]. However, in a Bangladesh study, all of the 10 metronidazole benzoate suspension samples analyzed complied with pH limits while two failed in assay [7]. Elsewhere, in South and West Yemen, ultraviolet (UV) spectrophotometric analysis of nine brands of metronidazole benzoate suspensions yielded 44.4% non-compliance with British Pharmacopoeia (BP) specifications for pH and assay [8]. In a similar study conducted in Warri, Nigeria involving 10 brands of metronidazole benzoate suspensions, nine complied with assay and eight with pH limits of the British Pharmacopoeia [9].

In Kenya, published reports so far have covered the quality of metronidazole benzoate suspensions in the context of quality control studies of samples submitted to specific laboratories [10, 11]. This is the first quality surveillance study of metronidazole benzoate suspensions marketed in Nairobi County, Kenya.

MATERIALS AND METHODS

Samples

A total of 32 samples comprising 13 brands were collected from retail pharmacies in Nairobi County during the months of March - April 2021, through convenient sampling. Samples were obtained from nine locations including Embakasi, Umoja, Mathare, Eastleigh, Kawangare, Westlands, Kibera, Lang'ata and the Central Business District (CBD). Up to three batches per brand were sampled from different sites. The samples were stored under ambient conditions until analysis. Quality control was carried out according to BP (2017) specifications for pH, metronidazole limits and assay [12].

Materials, reagents and solvents

HPLC grade methanol, ammonium acetate (Loba Chemie PVT Ltd, Mumbai, India), ammonia solution 25% w/v (RFCL Ltd, Haryana, India) were used to prepare mobile phases. Metronidazole (99.40% w/w) and metronidazole benzoate (99.38% w/w) working reference standards were kind donations from Laboratory and Allied Limited, Nairobi. Macherey-Nagel filter papers (Düren, Germany) and PTFE 0.22 µm microfilters (Nantong Filter-Bio Membrane Co., Jiangsu, China) were used in the filtration of analytical solutions.

Liquid Chromatograph system

A high-performance liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) was used for identification and assay of the samples. It consisted of a LC-20AD solvent delivery mechanism, a SIL-20A HT Prominence autosampler supported by a CBM-20A communication bus module and Shimadzu LC Solution software. Eluents were monitored by means of a SPD-20A UV/Visible detector set at 310 nm. Separation was achieved using Gemini® NX, C18 column of dimensions 250 mm × 4.6 mm ID, particle size 5 µm (Phenomenex Inc, Torrance, CA, USA) maintained at 30 °C by means of a CTO-10AS VP oven. The mobile phase was pumped isocratically at a flow rate of 1.0 ml/min.

pH meter

Neat samples were subjected to pH measurement on a TitroLine® 6000 titrator (SI Analytics GmbH, Mainz, Germany), equipped with VGA TFT display operating in the pH module. The equipment was calibrated in the 4.0 - 7.0 pH range using buffer solutions.

Mobile phases

The mobile phase consisted of methanol - 1.25% w/v ammonium acetate buffer (60:40). The pH of the buffer was adjusted to 7.0 using 6 M ammonia solution. Mobiles phases were degassed by means of a MRC DC 200H ultrasonic bath (Holon, Israel) for 15 minutes before use.

Standard Preparation

Metronidazole (200 mg) and metronidazole benzoate (320 mg) working reference standards were weighed into a 250 ml volumetric flask and dissolved in 150 ml methanol under ultrasonication for 10 minutes. The resulting mixture was topped up with distilled water, whereof five ml of the solution was diluted to 50 ml with mobile phase and microfiltered prior to injection.

Sample Preparation

Metronidazole benzoate suspension samples were each sonicated for 10 minutes and weight per ml determined using a 25 ml pycnometer. Thereafter, five grams of the suspension was weighed into a 250 ml volumetric flask, 150 ml methanol added and ultrasonicated for 10 minutes. The mixture was made to volume with distilled water and passed through filter paper. A five ml aliquot of the filtrate was diluted to 50 ml and microfiltered before chromatography.

RESULTS AND DISCUSSION

Table 1 is a summary of the pH, metronidazole content and assay results obtained for the metronidazole benzoate samples analyzed. Samples were coded using numerals (with prefix M) for unique brands and letter designations A, B, C for different batches of the same brand. The originator brand product M1 was manufactured in South Africa while all generics (M2-M13) were locally produced. Brand M1 was packaged in 100 ml glass bottles while the rest of the products were presented in 60 ml or 100 ml plastic bottles. All containers were amber coloured.

The results show that only nine samples (28.1%) complied with all tests carried on the samples. Conversely, five (15.6%), seven (21.9%) and 18 (56.3%) samples did not comply with BP specifications for pH, metronidazole limits and assay respectively. Five samples failed in more than test as recorded in Table 1.

Four brands; M3, M4, M10 and M13, had all batches analyzed failing assay while for the rest, one or two out of three batches were non-compliant in this test. All the non-compliant samples were found to contain active pharmaceutical ingredient (API) content below the lower limit [12]. Metronidazole benzoate is a prodrug that readily undergoes de-esterification in the gastrointestinal tract to form metronidazole, which appears in circulation. Low assay values may therefore cause sub-therapeutic blood concentrations of active drug following oral administration. Assay is a critical quality attribute that must be controlled for product consistency. Assay non-compliance is characteristic of substandard and falsified medicines (SFM) which affects medication effectiveness with adverse patient outcomes. The impacts of SFM include morbidity and mortality, resistance to antimicrobials, loss of confidence in health systems and economic losses [13].

In this study 21.9% of the samples had metronidazole content exceeding limits. Metronidazole is a hydrolysis degradation product of metronidazole benzoate during shelf life. This is likely to occur in cases of poor product design that predisposes the products to deterioration. A high metronidazole level is likely to modify the taste of the product thus undermining compliance among users. This degradation is acid/base catalyzed, with a stable pH range of 4-5, while alkaline pH yields faster reaction rates [5]. Incidentally, the samples that did not comply with pH recorded values higher than 6.5 which could cause API hydrolysis.

Table 1: Analytical results of metronidazole benzoate suspension samples obtained from Nairobi County

Sample code	Pack size (ml)	pH (5.0-6.5)	Metronidazole (NMT 1.0%)	Assay (95.0 – 105.0 % LC)	Tests failed
M1A	100	6.1	0.3	97.4 (0.1)	-
M1B	100	6.1	1.1	99.2 (0.2)	M
M2A	60	5.4	0.4	99.7 (0.1)	-
M2B	60	5.3	0.2	97.2 (1.3)	-
M2C	60	5.3	0.1	99.8 (0.5)	-
M3A	100	6.2	0.2	93.8 (0.1)	A
M3B	60	5.2	0.3	92.4 (1.1)	A
M4A	60	6.0	0.5	94.0 (0.2)	A
M4B	60	7.0	1.2	93.4 (0.1)	P, M, A
M4C	60	5.5	0.3	92.1 (0.4)	A
M5A	60	6.0	1.9	90.0 (0.1)	M, A
M5B	100	4.5	0.1	96.7 (1.1)	-
M5C	60	6.7	1.6	84.7 (0.0)	P, M, A
M6A	100	6.2	0.3	93.1 (0.2)	A
M6B	100	6.8	0.3	99.7 (1.0)	P
M6C	60	7.3	0.8	94.6 (1.0)	P, A
M7A	100	5.1	0.8	92.3 (0.1)	A
M7B	100	5.5	0.1	95.2 (0.1)	-
M7C	100	5.4	0.9	97.9 (0.5)	-
M8A	60	5.4	0.2	93.1 (0.6)	A
M8B	100	5.8	0.2	100.0 (0.3)	-
M8C	100	5.6	0.3	88.0 (0.2)	A
M9A	100	5.9	0.2	92.3 (1.7)	A
M9B	60	5.7	0.3	92.3 (0.3)	A
M9C	60	6.2	2.7	103.9 (0.4)	M
M10A	100	5.5	0.2	89.6 (0.1)	A
M11A	60	5.8	0.4	88.6 (0.1)	A
M11B	60	6.7	1.7	97.4 (1.8)	P, M
M11C	100	5.8	0.3	95.5 (0.1)	-
M12A	100	5.4	0.1	92.5 (0.7)	A
M12B	100	5.1	1.6	98.8 (0.2)	M
M13A	60	5.8	0.2	94.5 (1.6)	A

LC - label claim, NMT - not more than. Figures in parentheses represent coefficient of variation.

Drug product quality is determined by product design, manufacturing process, plant environment, quality control system and distribution practices. In order to consistently produce products of suitable quality, manufacturers should institute a quality assurance framework supported by current good manufacturing practices (cGMP) and quality management system (QMS) [14, 15]. This employs principles of quality by design

(QbD), which greatly reduces the risk of producing substandard products [16]. Furthermore, implementation of quality risk management principles minimizes quality defects through adequate process control [15]. It is therefore imperative that manufacturers embrace these practices to ensure that quality, safety and efficacy of medicines released to the market is maintained. Drug regulatory authorities on the other hand, bear the responsibility of enforcement of quality standards through market authorization, cGMP audits, pharmacovigilance and post market surveillance programmes. Furthermore, appropriate regulatory actions against perverse players are essential to protect patients from SFM.

CONCLUSION

A quality survey of metronidazole benzoate suspension products was carried out in Nairobi County. The samples obtained were subjected to the BP (2017) specifications for pH, metronidazole content and assay whereof the overall compliance rate was 18.1%. The results obtained demonstrate the existence of SFM in the market which may yield adverse patient treatment outcomes. Pharmaceutical production for this class of medicines should follow cGMP guidelines to limit the prevalence of poor quality products in circulation. Moreover, regular post market surveillance surveys are necessary to reveal the extent of this problem and elicit appropriate regulatory actions.

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