Analysis of Co-trimoxazole Products on the Kenyan Market

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The content of Sulphamethoxazole/Trimethoprim preparations were evaluated using the official British Pharmacopoeia method and a liquid chromatographic method. The two methods were shown to give similar performances and were used alternately. Twenty two tablet samples from 16 products were analysed of which 7 failed the requirements of content. Fifteen Co-trimoxazole suspension samples from 11 products were analysed of which 4 failed the content requirements. There were variations in chemical content amongst batches of same product. Failure rate amongst samples was 31% for imported and 22% for locally manufactured tablets and 50% for imported and 11% for locally manufactured suspensions.

Key Words: Co-trimoxazole, Quality Assurance, Analysis.

INTRODUCTION

The combination of the two bacteriostatic drugs sulphonamide and trimethoprim affords a bactericidal product due to sequential block and hence synergistic action. Products of these combinations are extensively used.

The most common combination product is Co-trimoxazole which contains Sulphamethoxazole (SMZ) and Trimethoprim (TMP) in an optimal ratio of 5:1. Cotrimoxazole appears in the Ministry of Health Kenya [1], and World Health Organisation (WHO) [2] lists of essential drugs for use at all levels of health care and in those recommended for the management of some sexually transmitted diseases [3].

The combination product has high patient compliance because of the dosing convenience of every twelve hourly. Further, the combination does not have distressing gastrointestinal tract effects.

Emerging resistance to Co-trimoxazole has been reported [4,5]. Contributing to this problem is exposure to sub-therapeutic levels occasioned by low dose intake consequent to poor quality. This could further be compounded by the ease of obtaining of Co-trimoxazoles without prescription [6].

The quality of Co-trimoxazole products on the Kenyan market has been mentioned periodically in publications [7-9]. These publications have however reported only on the quality of those samples analysed on request from either the Government or other agencies including individuals. A non-compliance level of about 20% was reported.

Analysis of Co-trimoxazole may be done following the official Compendial methods [10, 11]. However, some manufacturers also use in-house liquid chromatography (LC) methods as alternatives.

The aim of the present study was to investigate the quality of Co-trimoxazoles preparations on the Kenyan market with special emphasis on chemical content as determined by the official BP method [10] and a LC method. This paper reports on the quality of Co-trimoxazole samples collected from the market and analysed over a period of 3 years.

MATERIALS AND METHODS

Reference materials, samples and reagents

Chemical reference substances (BPCRS) of SMZ (Lot 5071) and TMP (Lot 1550) were obtained from the British Pharmacopoeia commission. Working standards of SMZ (99.7%) from Virchow Laboratories Ltd., India and TMP (98.8%) Chemphar, China were obtained courtesy of Cosmos Limited, Nairobi, Kenya and analysed against the BPCRS. Analytical grade salicylic acid from E.T. Monks, Nairobi, was used as an Internal Standard (IS).

Co-trimoxazole preparations evaluated were sampled and analysed during a three year period 1992-1995. Most samples were obtained from pharmacies in Nairobi. Others were samples submitted to the Pharmacy and Poisons Board, Ministry of Health, for the purpose of registration prior to marketing in Kenya. Details of local and imported products that were analysed is given in Tables 1 and 2 respectively. All products analysed had more than one third of the specified shelf life remaining. Declared shelf-life range was between 3 and 5 years.

Analytical grade ammonium acetate and acetic acid from BDH, Poole, UK were used to prepare 0.1 M Ammonium acetate buffer. Reagent grade methanol (BDH) and water were distilled from a glass apparatus.

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TABLE 1: Manufacturers of Local Products Analysed

Name of Product	Formulation	Manufacturer
Alprim Tabs	Tablets/suspension	Elys Chemical Industries Ltd.
Cosatrim	Tablets/suspension	Cosmos Ltd.
Maxotrim	Suspension	Mac's Pharmaceutical Ltd.
Trimoxol	Tablets/suspension	Dawa Pharmaceuticals Ltd.
Septrim	Tablets/suspension	Weilcome Ltd.
Unitrim	Tablets/suspension	Regal Pharmaceuticals Ltd.
Trizole	Suspension	Pharmaceutical Manufacturing Co. Ltd.
Laecotrim	Tablets/suspension	Laboratory and Allied Ltd.

TABLE 2: Manufacturers of Imported Products Analysed

Name of Product	Formulation	Manufacturer
Ultrasept	Tablets	Teva, Tel Aviv, Israel
Co-trimoxazole	Tablets	S. Kant, Bombay, India
Co-trimoxazole	Tablets	Helm, Germany
Apo-sulfatrim	Tablets	Apotex, Canada
Parkazole	Tablets	Parke-Davis
Co-trimoxazole	Tablets	Pharmamed, Cyprus
Trizole	Suspension	Pharmamed, Cyprus
Co-trimoxazole	Tablets	Rivopharm, Switzerland
Sumetrolim	Tablets	Egis Pharma, Hungary
Oriprim	Tablets	Cadila, Bombay - India
Scanprin	Suspension	Denken, Denmark
Co-trimoxazole	Tablets	Meghdoot Chemicals, India

Methods of Analysis

The BP method for the determination of the SMZ and TMP content in Co-trimoxazole products uses respectively the procedures of diazotization and ultraviolet spectrophotometry. Further, tablet products were evaluated for uniformity of weight, hardness and disintegration times using BP procedures [10].

A LC method for the simultaneous analysis of SMZ and TMP in Co-trimoxazole was used. The LC method was modified from that described in the USP [11]. Methanol replaced the more expensive acetonitrile. Salicylic acid was used as the Internal Standard (IS). The method afforded good separation of SMZ, TMP and IS with a chromatographic run time of 20 minutes.

The liquid chromatographic system consisted of model L6200 solvent delivery system and model L4200 detector set at 230nm (Merck, Darmstadt, Germany), model CV-6-UHPa-N60 sample injection valve (Valco, Houston, TX, USA) equipped with 25µ loop and 3396 series II integrating recorder (Hewlett Packard, Avondale, NY, USA). The column, 250 x 4.6 mm, was laboratory packed with RSil C_{18}HL 10µm (Biorad, Eke, Belgium) and was maintained at 40°C by immersion in a water bath.

Mobile phase: methanol-water-0. 1M ammonium acetate buffer adjusted to pH 5.0 with 0. 1M acetic acid (25:65:10). The mixture was degassed by sonication before use. Flow rate was set at 1.0 ml per minute.

A working standard as well as test sample solutions were prepared by accurately transferring an amount of sample equivalent to 400 mg SMZ and 80 mg TMP into a 250 ml volumetric flask, 10.0 ml of 0.1 M NaOH and 50 ml methanol were added. The mixture was dissolved by sonication and made up to volume with water. The mixture was filtered through a 0.45µm membrane filter, 1.0 ml of the filtrate was pipetted into a 50 ml volumetric flask containing 1.0 ml of internal standard and the mixture made up to volume with water. 25µL of solution was injected three times and the mean peak area ratio was calculated.

Five point calibration curves were obtained with SMZ and TMP working standards. Each point was an average of three independent analyses. The following relationships were found, where CR = range of injected mass examined and r = correlation coefficient. Sulphamethoxazole: CR = 640-960 µg, r = 0.9952 and Trimethoprim: CR = 128-192 Pg, r = 0.9980.

The percentage content of SMZ and TMP in the samples was calculated by comparing the peak area ratios for the sample with that of the working standard, obtained the same day.

RESULTS

Galenical Evaluation of Co-trimoxazole Tablets

Galenical evaluation of tablet products for uniformity of weight, hardness and disintegration times showed them to comply with compendial requirements except for product VIII which gave disintegration times longer than 15 minutes.

The BP and LC methods were compared by analysing the same sample nine times using each method. The results are shown in Table 3. Both methods give equivalent results.

Decision on whether a product met the requirement for chemical content was based on the analysis method that gave results closer to the limits specified. The results of chemical content of tablet preparations are shown in Table 4. There was a total of 9 samples from 7 local products of which 2 failed the content. Of the 9 imported products, 13 samples were analysed and 4 failed in content specifications. Six samples from four products failed the specifications for content of active ingredients. The content of SMZ was low in samples IVc and XIc, and high in XIb. For products IV, XI and XII where more than one batch was analysed, high batch variations were observed for the active ingredients. The LC assay was used for 13 samples. VII and XIXb had a content of TMP exceeding 107.5% while it was less than 92.5% for III.

Results obtained for suspensions are shown in Table 5. Following the BP method of analysis, all products had SMZ and TMP contents within the BP limits of ±10% of label claim. The LC analysis of eight samples shows three samples to have a sulphamethoxazole content lower than 90%. The Trimethoprim content was within limits for all samples except VII b with a content of 84.4%. A total of 16 samples were analysed. Nine samples were from 7 products of local origin and only one failed to meet the content requirements. Meanwhile, 6 samples from 4 imported products were analysed of which 3 failed.

TABLE 3: Content* of Sulphamethoxazole and Trimethoprim Obtained on Same Sample With B.P.; and LC Methods

Method	Sulphamethoxazole content (%)			Trimethoprim content (%)		
	Range	Mean	RSD	Range	Mean	RSD
B.P.	98.9-101.3	99.3	0.9	95-103.9	98.8	2.58
LC	97-100.6	98.4	1.1	96.4-101.3	98.6	1.78

*n = 9 determinations

TABLE 4: Chemical Content* of Co-trimoxazole Tablets as Determined Using the BP and LC Methods

Product		B.P.		LC	
		SMZ	TMP	SW	TMP
I		101.8	101.1	-	-
II		-	-	101.6	101.1
III		-	-	96.6	86.9
IV	a	97.1	96.0	95.4	93.3
	b	96.3	138.7	101.8	95.3
	c	79.1	93.5	-	-
V		101.4	98.1	102.9	103.7
VI		96.2	101.2	102.3	104.0
VII		98.4	96.9	104.1	108.1
VIII		101.0	106.2	-	-
IX		99.9	101.6	-	-
X		104.9	102.3	-	-
XI	a	99.1	97.4	103.8	104.0
	b	95.9	79.7	-	-
	c	69.4	100.1	-	-
XII	a	103.3	100.5	-	-
	b	94.9	137.5	-	-
XIII		-	-	99.4	98.0
XIV		117.1	130.8	97.5	97.5
XVI		-	-	102.7	103.4
XIX	a	-	-	103.2	104.2
	b	-	-	102.4	110.8

*BP. Limits for both ingredients, 92.5 - 107.5 of stated amount, n = 3 independent analysis
SMZ = Sulphamethoxazole, TMP = Trimethoprim
a, b, c, etc refer to batches

TABLE 5: Chemical Content* of Co-trimoxazole Suspensions as Determined Using the B.P. and L.C. Methods

Product		B.P.		LC	
		SMZ	TMP	SMZ	TMP
I		98.3	98.4	-	-
II	a	104.1	104.9	-	-
	b	102.0	104.9	-	-
	c	99.6	104.1	-	-
III		99.2	104.2	99.5	94.4
IV		104.1	105.2	79.1	93.5
V		-	-	96.9	93.6
VII		102.8	108.6	-	-
XI		-	-	69.4	100.1
XII	a	99.5	91.9	-	-
	b	104.2	102.9	-	-
XV		104.9	06.5	-	-
XVII		-	-	69.8	ND
XVIII	a	-	-	100.4	101.0
	b	-	-	91.6	84.4

* BP. Limits for both ingredients, 90 - 110% of stated amount, n = 3 independent analysis

SMZ = Sulphamethoxazole, TMP = Trimethoprim ND = Not determined,

a, b, c, etc refer to batches

DISCUSSION

An analytical method is a critical tool during preparation and quality control of dosage forms. Quality control objectives are better addressed with a method that is more selective such as the LC. A number of analysis on the same sample were done by both LC and BP methods. On the average, the BP method afforded slightly higher content values. The result would suggest that LC be used more as it affords good separation of SMZ and TMP during a single run. The analysis time required to carry out the LC method is also very short. This allows for faster analytical throughput during production.

The content of active constituents in tablets and suspensions are as shown in table 4 and table 5 respectively. The content of active component range from a low of 69.4% to a high of 138.7% of labelled amount. Whilst a higher content than allowed may not pose problems of side effects for such products, those with low amounts could lead to sub-therapeutic levels. Such levels in blood contribute significantly to the observed emerging microbial resistance to Co-trimoxazole. For three tablet products there were observed content variation amongst batches. The variations are indicative of possible problems with observance of good pharmaceutical manufacturing practices. Some batches of products IV and XI had ingredient contents much outside the compendial limits. Other products could also be having similar problems except that only one batch each was evaluated.

A number of products were analysed using both the BP and LC methods. The results show a higher chemical content with LC than BP method and vice-versa. This could be due to interferences from excipients and/or related substances. Manufacturers use different excipients some of which could interfere with either method. Therefore, selection of a method to use during a market surveillance need careful thought. Method application may require modifications to handle some products.

Those products whose content was within limits with either method of analysis are treated as having met the requirements. From the study, 69% of imported and 78% of locally prepared tablet samples met the compendial requirements for the content of active ingredients. The pass rate for suspensions was, imported - 50% and local - 89%.

Kenya is a malaria endemic region. "Fansidar" type of product that contain Sulphadoxine and other Sulphas are readily available for management of malaria. In most cases self diagnosis of malaria and liberal use of sulphadoxine is very common. It is possible that Sulphadoxine has cross-resistance with sulphamethoxazole hence contributing to resistance to Co-trimoxazole. The converse could also be true with serious implications for the malaria control programme in the country. Cross-resistance between sulphadoxine and sulphamethoxazole could be part of an explanation for the question posed by Ndinya-Achola *et. al.* [12] in regard to more than expected microbial resistance to Co-trimoxazole.

The quality of sulphadoxine/pyrimethamine products is currently under investigation, since these products are now being increasingly used as first line treatment for Malaria. Market surveillance of Co-trimoxazoles and other antimicrobial agents would help remove substandard products from the market as part of the fight against emerging microbial resistance.

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