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Composition of Gentamicin C Components in Gentamicin Sulphate Generics Commonly Used in Small Animal Practice in Nigeria

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Abstract: Gentamicin is one of the commonly used antibiotics in small animal practice in Nigeria. Fake and substandard drugs are responsible for high cost in both economic terms and lives lost. For decades, Nigeria has been flooded by counterfeit and poor-quality medicines. Because of the variations in gentamicin C components in different formulations and the effect of this on its efficacy and toxicity, this study was designed to evaluate the percentage of each of the major components of gentamicin C in some injectable gentamicin sulphate generics commonly used in small animal practice in Nigeria. Of the 22 multisource generics of injectable gentamicin sulphate samples analyzed for percentage content of gentamicin C major components using USP HPLC (United States Pharmacopoeia high performance liquid chromatography) method, 95.5% (21) met the USP specification. This suggests that there is a significant improvement in the monitoring of quality of drugs marketed in Nigeria, including gentamicin sulphate. Nevertheless, considering the propensity of the manufacturers adjusting their manufacturing processes following product's registration by the regulatory body, there is still the need for regular surveillance of drug products by batches to ensure their efficacy and safety.

Key words: Gentamicin, generics, multisource, small animals, Nigeria.

1. Introduction

Gentamicin is an aminoglycoside antimicrobial agent produced by the fermentation of *Micromonospora purpurea*. It is not a single compound but a complex of three major components, such as C_1, C_{1a} and C_2, as well as other minor components, including C_{2a} and C_{2b} (sagamicin) [1, 2]. In addition, other related substances, such as sisomicin, garamine, gentamicin B_1, and 2-deoxystreptamine are formed in small amounts during its manufacturing process [3]. Gentamicin is associated with severe side effects of which the most clinically significant are nephrotoxicity and ototoxicity [4, 5]. Gentamicin components differ in their antimicrobial potencies and toxicity in animals [6, 7]. There have been reports of significant differences in disposition profiles between gentamicin major components in dogs [8], horses [9], turkeys [10] and chickens [11]. It has also been reported that there is a wide variation in the major component ratio between different pharmaceutical gentamicin products [12, 13]. Consequently, the USP (United States Pharmacopoeia) has set a limit of 25~50% for C_1, 10~35% for C_{1a}, and 25~55% for the sum of C_2 and C_{2a} [1].

In 2002, the WHO (World Health Organization) reported that 70% of drugs in Nigeria were fake or substandard, whereas NAFDAC (National Agency for Food, Drug Administration and Control) estimated that 41% of drugs were counterfeit [14]. Again, there is paucity of information on the components ratio variations in the arrays of multisource commercial

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formulations of gentamicin sulphate marketed in Nigeria. Thus, this informs the need to routinely investigate and control the ratio of major components of gentamicin C, as well as related substances in these commercial products. Consequently, this study was primarily designed with the purpose of evaluating the percentage of each major component of gentamicin C in some injectable gentamicin sulphate generics commonly used in small animal practice in Nigeria.

2. Material and Methods

2.1 Chemicals and Drugs

USP gentamicin sulphate reference standard (USP Rockville, Lot No. MOD314) was used to obtain the reference peak for each gentamicin component as described [1]. The pre-column derivatizing reagents used were HPLC (high performance liquid chromatography) grade from Sigma-Aldrich, Germany, such as isopropanol (Lot No. K46024734-447), boric acid (Lot No. SZBE1610V), potassium hydroxide (Lot No. SZBE3440V), phthaldialdehyde (Lot No. BCNN6399V) and thioglycolic acid (Lot No. STB3702V). In addition, sodium heptane sulfonate, an ion-pairing agent (Sigma-Aldrich, Switzerland, Lot No. BCBP7095V) and glacial acetic acid (Merck KGaA, Germany, Lot No. K1722963-135) were used. Generics of injectable gentamicin sulphate were purchased from different veterinary and pharmaceutical stores in Nigeria.

2.2 Reagents and Sample Preparations

Reagents and samples were prepared to obtain their respective final concentrations as specified in the USP [1, 14]. Solution of potassium hydroxide (8 M) was prepared by dissolving 44.9 g of the powder in 100 mL distilled water (Solution 1). Boric acid buffer (0.4 M) was prepared by dissolving 4.94 g of the acid in 180 mL of distilled water and adjusted to apparent pH (pH*) of 10.4 with Solution 1, making the final volume to 200 mL (Solution 2). Thereafter, 1 g of phthaldialdehyde was weighed into a 250 mL beaker and dissolved with 5 mL of methanol (Solution 3). To Solution 3 was added 95 mL of Solution 1 followed by 2 mL thioglycolic acid. The pH* (10.4) was maintained using Solution 2.

Accurately, 20 mg of gentamicin sulphate reference standard (USP) was weighed and dried under a vacuum. This was subsequently dissolved in 6.9 mL of distilled water to obtain 2 mg/mL free base reference standard solution as specified by the manufacturer. Similarly, 2 mg/mL of each generic of injectable gentamicin sulphate (n = 22) was prepared. Pre-column derivatization was done by adding 440 µL of isopropanol and 160 µL of the derivatizing reagent (Solution 3) into a 2 mL auto-sampler vial containing 0.4 mL of the sample and vortexed for 10 s. This was subsequently subjected to heat at 60 °C in a heating block for 15 min and allowed to cool to room temperature before transferring to the auto-sampling chamber in the HPLC.

Ion-pairing mobile Phase A was prepared by dissolving 5 g sodium heptane sulfonate in 250 mL of distilled water. Thereafter, 50 mL glacial acetic acid was added and made up to 1 L with methanol. Whereas ion-pairing mobile Phase B was prepared by dissolving 0.5 g sodium heptane sulfonate in 300 mL distilled water followed by 60 mL methanol, then 5 mL glacial acetic acid.

2.3 Instrumentation and Condition

A Hewlett Packard series 1100 HPLC (Agilent 1260 Infinity Agilent Technologies, Germany) equipped with an ultraviolet-visible detector (G1314B) of variable wavelength, quaternary pump (G1311A), auto-sampler with an injection loop, column thermostatic compartment, degasser (G1379A), as well as Agilent Chem Station software for data acquisition was used. The chromatographic column used was Interchrom HS Strategy-3-RP-C18 (Interchim Technology, France) with particle size 3 µm and dimension 50 mm × 2.0 mm attached to a guard. The column was set at ambient temperature (25 °C) and the

quaternary pump was used to deliver the mobile phase at a flow rate of 0.25 mL/min. The auto-sampler was programmed to inject a sample volume of 5.0 μL at the completion of each run of 22 min. Furthermore, the chromatographic eluents were monitored at a detection wavelength of 330 nm. Thereafter, results were printed to record the respective peak area responses.

2.4 Assay

The analysis was done employing the method reported by USP [15] and Kuehl et al. [16]. The mobile phase was made up of methanol (700 mL), distilled water (250), glacial acetic acid (50 mL) and Sodium 1-heptanesulfonate (5 g).

2.5 Calculation of the Percentage Components Content

The percentage contents of gentamicin C_1, C_{1a}, C_{2a}, C_{2b} and C_2 in the each of the gentamicin sulphate generic was computed employing the formula [14]:

$$\text{Percentage component} = \frac{r_f}{r_s} \times 100$$

where, r_f is the peak area response corresponding to the particular gentamicin component, and r_s is the sum of the area responses of gentamicin C_1, C_{1a}, C_{2a}, C_{2b} and C_2. These values were thereafter compared with the current official USP monograph for gentamicin sulphate injectable formulation [1].

3. Results

Several multisource injectable gentamicin sulphate samples (8 veterinary and 14 human formulations) were investigated using a slightly modified USP HPLC technique in this study [1]. Figs. 1 and 2 represent liquid chromatograms of the blank mobile phase and USP reference standard of gentamicin sulphate, respectively.

The elution order of the components based on the adopted method was gentamicin C_1 (4.5 min), C_{1a} (10.4 min), C_{2a} (12.7 min), and C_2 (14.4 min). Liquid chromatograms for the generic that failed the test and a representative of the ones that passed the test are presented in Figs. 3 and 4, respectively.

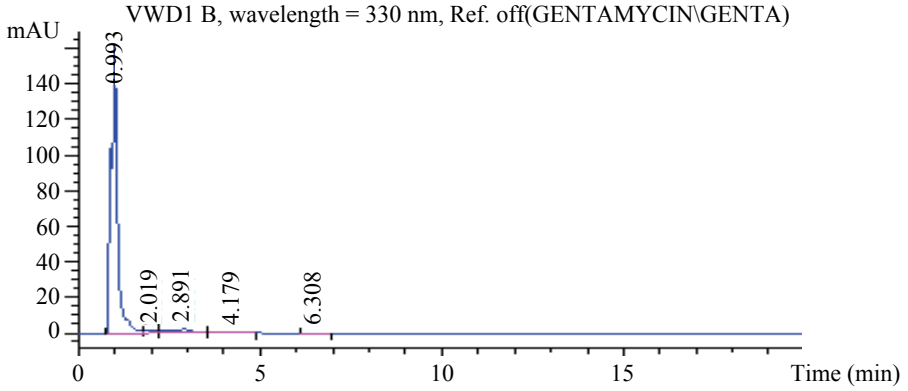

Fig. 1 Liquid chromatogram of blank ion-pairing mobile phase.

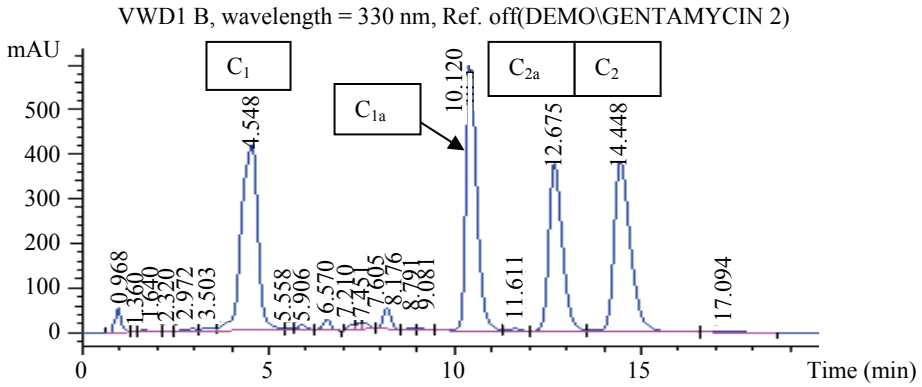

Fig. 2 Liquid chromatogram of USP gentamicin sulphate reference standard.

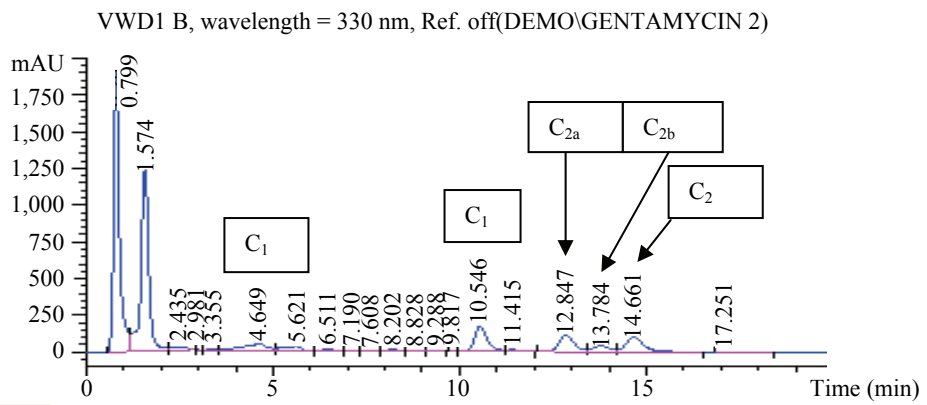

Fig. 3 Liquid chromatogram of gentamicin sulphate (generic T) showing non-interference from other active ingredients on gentamicin components peaks at 4.6, 10.5, 12.8, 13.8 and 14.6 min.

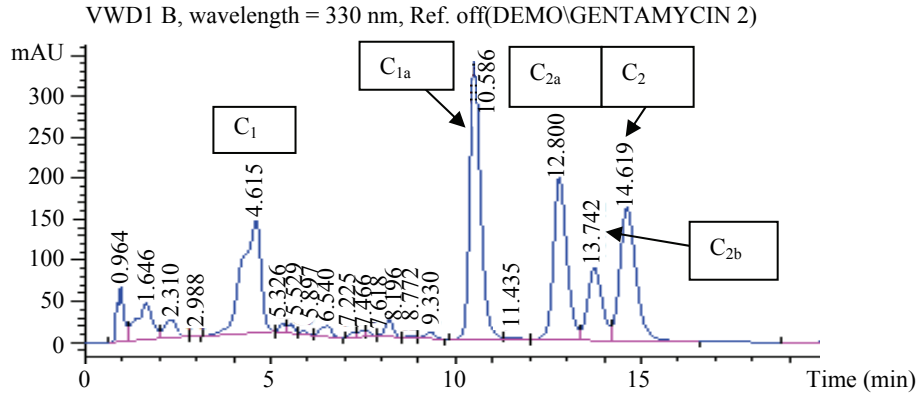

Fig. 4 Liquid chromatogram of gentamicin sulphate (generic U) showing non-interference from other active ingredients on gentamicin components peaks at 4.6, 10.5, 12.8, 13.7 and 14.6 min.

The computed percentages of each of the major components in the reference standard, as well as the 22 generics of injectable gentamicin sulphate as required by the USP monograph for gentamicin sulphate [1] are presented in Table 1.

4. Discussion and Conclusions

The number of components and impurities in gentamicin complex make its chromatographic analysis not quite straightforward [2, 17]. Furthermore, gentamicins are basic, highly polar, relatively stable and structurally closely related compounds without UV (ultraviolet) and fluorescence absorbing chromophores and fluorophores, respectively [18, 19]. These properties make HPLC analysis of gentamicin more difficult and challenging necessitating the need for derivatization of amino groups with derivatizing reagents, such as phthaldialdehyde, 2,4,6-trinitrobenzensulfonic acid and 1-fluoro-2,4-dinitrobenzene that allow for UV [19, 20] or fluorescence detection [21]. In this study, phthaldialdehyde (pre-column derivatizing agent) improved the chromatographic behavior of gentamicins making them less polar, introducing a chromophore in the molecule and subsequently been absorbed and detected by the UV detector. Also, sodium heptane sulfonate was used as an ion-pairing agent and it satisfactorily improved the separation of gentamicin components in the HPLC analysis. This enabled the circumvention of the common challenge of separating gentamicin related compounds under reverse phase HPLC conditions [22]. The mobile phase and excipients in the formulations did not interfere with gentamicin peaks as depicted in the chromatograms. All the peaks representing gentamicin major components in the USP reference standard and generic samples resolved adequately. In addition, the peaks obtained from the generic samples

Table 1 Relative composition of major gentamicin components in some commercial injectable formulations commonly used in Nigeria.

Sample	Percentage content of major components (%)		
	$C_1 + C_{2b}$	C_{1a}	$C_2 + C_{2a}$
URS	28	26	46
A	31	21	48
B	32	30	38
C[a]	31	27	42
D	50	23	27
E[b]	27	25	48
F[b]	27	25	48
G	32	24	44
H	32	28	40
I	39	26	35
J	28	22	50
K	29	27	44
L	27	24	49
M	27	27	46
N*	31	32	37
O*	33	25	42
P*[a]	31	27	42
Q*	31	30	39
R*	30	30	40
S*	33	27	40
T*	24[#]	29	47
U*	30	29	41
V	31	31	38
USP specification	25~50	10~35	25~55

URS: USP reference standard;
[a, b] same superscript indicates similarity in percentage components constituent;
* veterinary formulation;
[#] failed test.

were comparable with those observed in the USP reference standard.

According to the current specification, relative percentage of the sum of gentamicin C_1 and C_{2b}, gentamicin C_{1a}, as well as the sum of gentamicin C_{2a} and C_2 must be within the limits 25~50%, 10~35% and 25~55%, respectively [16]. The result demonstrated that 21 (95.5%) of the generic samples met USP specifications. However, only generic sample coded T failed the test since the percentage content of $C_1 + C_{2b}$ was 24% which is below 25% specified in the USP. Thus, the relative compositions of the major gentamicin components were observed to be similar in generic samples E and F (27%, 25% and 48% for gentamicin $C_1 + C_{2b}$, C_{1a} and $C_2 + C_{2a}$, respectively), as well as C and P (31%, 27% and 42% for gentamicin $C_1 + C_{2b}$, C_{1a} and $C_2 + C_{2a}$, respectively).

This study demonstrates that there is a significant improvement in the fight against circulation of counterfeit and substandard drug products, particularly, injectable gentamicin sulphate formulation in Nigeria. Nonetheless, the propensity of manufacturers to adjust their production processes from time to time requires regular monitoring of drug products, including gentamicin sulphate to avoid the dangers associated with poor quality of drugs in humans and animals.

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References

[1] USP (United States Pharmacopoeia). 2013. *Gentamicin Monograph. USP29-NF24.* Rockville: United States Pharmacopoeia Convention Inc.

[2] EP (European Pharmacopoeia). 2014. *Gentamicin 8.1.* Strasbourg: Council of Europe, 2326-82.

[3] Wagman, G. H., Testa, R. T., and Marquez, J. A. 1970. "Antibiotic 6640. II. Fermentation, Isolation and Properties." *Journal of Antibiotics* 23: 55-8.

[4] Leehhey, D. J., Braun, B. I., Tholl, D. A., Chung, L. S., Gross, C. A., Roback, J., A., et al. 1993. "Can Pharmacokinetic Dosing Decrease Nephrotoxicity Associated with Aminoglycoside Therapy." *Journal of American Society of Nephrologists* 4: 81-90.

[5] Jager, P., and Van Altena, R. 2002. "Hearing Loss and Nephrotoxicity in Long-Term Aminoglycoside Treatment in Patients with Tuberculosis." *International Journal of*

Tuberculosis and Lung Diseases 6: 622-7.

[6] Kohlhepp, S. J., Loveless, M. O., Kohnen, P. W., Houghton, D. C., Bennet, W. M., and Gilbert, D. N. 1984. "Nephrotoxicity of the Constituents of the Gentamicin Complex." *Journal of Infectious Diseases* 149: 605-14.

[7] Stypulkowska, K., Blazewicz1, A., Fijalek, Z., and Sarna, K. 2010. "Determination of Gentamicin Sulphate Composition and Related Substances in Pharmaceutical Preparations by LC with Charged Aerosol Detection." *Chromatographia* 72: 1225-9.

[8] Isoherranen, N., Lavy, E., and Soback, S. 2000. "Pharmacokinetics of Gentamicin C_1, C_{1a} and C_2 in Beagles after a Single Intravenous Dose." *Antimicrobial Agents and Chemotherapy* 44: 1443-7.

[9] Steinman, A., Isoherranen, N., Ashoach, O., and Soback, S. 2002. "Pharmacokinetics of Gentamicin C_1, C_{1a} and C_2 in Horses after Single Intravenous Dose." *Equine Veterinary Journal* 34: 615-8.

[10] Shem-Tov, M., Gabor, N., Suth, M., and Kormoczy, P. 2003. "Depletion of Gentamicin and Its Major Components from Various Tissues of Turkeys." *American Journal of Veterinary Research* 64: 1234-6.

[11] Ehab, A. A., Ahmad, F. A., and Ronette, G. 2013. "Pharmacokinetics of Gentamicin C_1, C_{1a}, C_2 and C_{2a} in Broiler Chickens after IV, IM, SC and Oral Administration." *Journal of Bioequivalence and Bioavailability* 5: 129-35.

[12] White, L. O., Lovering, A., and Reeves, D. S. 1983. "Variations in Gentamicin C_1, C_{1a}, C_2, and C_{2a} Content of Some Preparations of Gentamicin Sulphate Used Clinically as Determined by High-Performance Liquid Chromatography." *Therapeutic Drug Monitoring* 59: 123-6.

[13] Claes, P. J., Busson, R., and Vanderhaeghe, H. 1984. "Determination of the Component Ratio of Commercial Gentamicin by High-Performance Liquid Chromatography Using Pre-column Derivatization." *Journal of Chromatography* 298: 445-57.

[14] Yankus, W. 2006. "Counterfeit Drugs: Coming to a Pharmacy near You." American Council on Science and Health. Accessed March 10, 2014. http://www.asch.org/publications/pubID.1379/pub_detail.asp.

[15] USP (United States Pharmacopoeia). 2007. *Monograph for Gentamicin Sulfate: USP29-N24.* Rockville: USP Convention Inc.

[16] Khuehl, P. J., De, S., Eppler, B., Marsters, J., Matthews, L., Reed, M. D., et al. 2012. "Development and Validation of an HPLC Assay for Dual Detection of Gentamicin Sulfate and Leucine from Novel Dry Powder for Inhalation." *J. Analytical and Bioanaytical Techniques* 3: 1-4.

[17] Waitz, J. A., Moss, E. L., Drube, C. G., and Weinstein, M. J. 1972. "Comparative Activity of Sisomicin, Kanamycin, and Tobramycin." *Antimicrobial Agents and Chemotherapy* 2: 431-7.

[18] Rok, G., and Lucija, Z. 2009. "Identification of Gentamicin Impurities by Liquid Chromatography Tandem Mass Spectrometry." *Journal of Pharmaceutical Biomedical Analysis* 50: 1037-43.

[19] Stead, D. A. 2000. "Current Methodologies for the Analysis of Aminoglycosides." *Journal of Chromatography B* 747: 69-93.

[20] Frutos, P., Torrado, S., Perez-Lorenza, M. E., Frutos, G. 2000. "A Validated Quantitative Colorimetric Assay for Gentamicin." *Journal of Pharmaceutical Biomedical Analysis* 21: 1149-59.

[21] Wang, H. Y., Liu, L. D., Sun, Y., Ma, L., and Li, J. 2000. "Determination of Gentamycin by Synchronous Derivative Fluorimetry." *Talanta* 52: 201-9.

[22] Joseph, A., and Rustum, A. 2010. "Development and Validation of RP-HPLC Method for the Determination of Gentamicin Sulfate and Its Related Substances in a Pharmaceutical Cream Using a Short Pentafluorophenyl Column and a Charged Aerosol Detector." *Journal of Pharmaceutical Biomedical Analysis* 51: 521-31.