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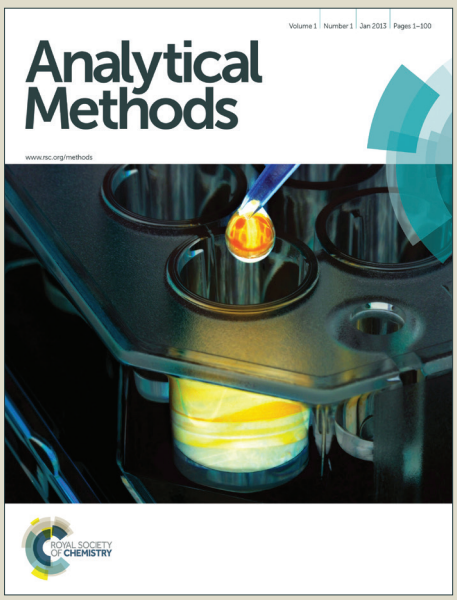

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Determination of thirteen antibiotics in drug products – a new LC-MS/MS tool for screening drug product quality

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Abstract

Poor quality antibiotic medicines in circulation in Sub-Saharan Africa continue to be a burden. Pharmaceutical trade in substandard and counterfeit medicines is on the rise. The chemical quality of antibiotics dispensed in health facilities and recognised drug outlets in Ghana, when compromised, could be a major drawback to efforts made in fighting antibiotic resistance globally. To improve on antibiotic drug quality monitoring, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology, which is capable of quantifying thirteen antibiotics in drug products, was developed and validated in present work. The methodology was applied to various drug products including tablets, capsules, suspensions, syrups, intravenous and injection solutions as well as ear and eye droplets used as essential medicines in a Sub-Saharan country, Ghana.

Keywords

Antibiotics; drug product quality; label guarantee; LC-MS/MS; sample preparation

1. Introduction

The quality of antibiotic drugs is of key importance in treatment of diseases of infectious origin and finds use in both human and veterinary applications. Insufficient duration of treatment, high costs of purchase and the occurrence and use of substandard and counterfeit antibiotic medicines in health facilities in Ghana and other developing countries in Sub-Saharan Africa remains a public health concern. The benefits and necessity of using high quality pharmaceutical products in health delivery systems cannot be overemphasised. However, drug quality can be compromised during manufacture, storage and distribution at elevated temperatures and humidity playing key roles in such processes [1]. *'Bitter than death'* to the fortunes of a country is the long-term circulation and usage of sub-standard antimicrobial agents in health delivery systems. Administration of poor quality anti-infectives for common, life-threatening and post-operative infections can be recipe for treatment failures, mortality, high cost for health care and development of microbial resistance. Consequently, public confidence in the health delivery system can be eroded with attendant loss of man-hours as a result of the disease burden of resistant microbial infections. Governments seeking to address the problem will have to spend huge sums of money without much guarantee for success.

Unfortunately, drug quality monitoring remains a challenge in most countries with emerging economies due to lack of facilities, poor regulatory mechanisms and lack of cost effective analytical methods [2-10]. As a result, the availability of sub-standard and fake anti-infectives in the drug supplies continues [11] with high incidence of diseases of microbial aetiology. The WHO continues to pay a significant level of attention to substandard and fake drugs [12-15] and it is reported that 15% of all drugs worldwide could be fake or substandard [16-22]. A WHO report on the quality of antibiotics in Ghana in 2010 indicated that 14% of the three product categories sampled and worked on were not of the desired quality [23] and similar or worse findings could be available in countries with similar socio-economic development as Ghana. A review of literature indicates that some assessment studies performed on the quality of antibiotics and antimicrobials in Africa mostly employed pharmacopoeial methods [2,3,23-25]. Notwithstanding the benefits of the pharmacopoeial methods, there are pertinent challenges with the

application of some of the procedures in resource-deprived environments. One of the key challenges is that, the pharmacopoeial methods are specific for particular active pharmaceutical ingredients (API) with requirements for a set of reagents, equipment and specialised analytical conditions that usually are not suitable for other APIs in the same or similar chemical classification. Where the assay method is biological, acquisition of standard reagents and facilities for reproducible and reliable results becomes a hurdle. Therefore, effectively applying the pharmacopoeial methods to control the quality of an array of antibiotic agents in a low-income country regularly can be capital, manpower and resource intensive. Most pharmacopoeia assay methods for dosage forms are based on liquid chromatography combined with UV detectors and in situations where adulterant(s) co-elute(s) or share(s) close UV absorption characteristics with the API, pharmacopoeia methods are unable to detect adulterants. The importance of the LC-MS/MS technique in pharmaceutical, forensic, proteomic, biological and environmental studies are well acknowledged [26-30]. There are reports of LC-MS/MS methods for identification and quantification of medicines, but most of these describe methods dealing with biological and environmental matrices [26,28-30] and not meant to be used as screening tool for the quality of antimicrobial pharmaceutical products. In this regard, the LC-MS/MS technique, which is capable of identifying and quantifying multiple chemical compounds, becomes a viable alternative. This study therefore aimed to develop two LC-MS/MS methods to determine 13 antimicrobial agents belonging to eight pharmacological classes in various pharmaceutical formulations for use as a screening tool for antibiotic quality monitoring. A complete list of the 13 investigated antimicrobial agents including chemical structure; name and physicochemical properties are shown in Table 1. The method being reported is cost-effective and sensitive with the potential for applications in therapeutic drug monitoring. It has the advantage that one method handles multiple compounds which usually is not possible with most existing methods. As a result, the burden of acquiring different sets of reagents and specialised conditions for various compounds is overcome. In addition, to the authors knowledge, LC-MS(/MS) instruments are becoming increasingly common installation in African universities and institutions. This makes it relatively affordable for countries south of Sahara to apply as a quality assurance tool for antimicrobial products.

2. Material and methods

2.1. Materials, Reagents and Standards

Analytical standards of penicillin G (PNG), ceftriaxone (CTX), tetracycline (TCC), gentamicin (GMC) and erythromycin (ETM) were purchased from Sigma-Aldrich (Glostrup, Denmark). Ampicillin (AMP), cefuroxime (CFX), ciprofloxacin (CPF), metronidazole (MTZ), calvulanic acid (CLV), trimethoprim (TMP) and sulphamethoxazole (STX) were obtained from Fluka (Glostrup, Denmark). Amoxicillin (AMX) was obtained from Duchefa Biochemie B.V. (Netherlands). All analytical standards had purity above 99%. All solvents used in the study including acetonitrile, methanol and buffer (formic acid/ammonium formate) were of analytical grade and purchased from Lab-Scan Analytical Sciences (Fischer Scientific Biotech Line, Denmark). MilliQ water (Millipore, Bedford, MA, USA) was used in all preparation of solutions. Mobile phase A was water:acetonitrile:methanol (90:6:4, v/v/v) and mobile phase B was water:acetonitrile:methanol (5:65:30, v/v/v). Both mobile phases contained 100 μL formic acid (98%), 100 μL triethylamine (≥ 99 %) and 0.35 g ammonium formate (≥ 99 %) per litre as common additives. Standard stock solutions of 1 mg/mL were prepared in the mobile phase mixture (A:B, 10:90, v/v). Commercially available antibiotic drug products of the compounds being studied were obtained from government hospitals, privately owned pharmacies, licensed chemical shops and peddlers (informal and unlicensed suppliers) in Ghana. Weight measurements were made using Sartorius MC5 and BP221S analytical balances.

2.2. Sample preparation

The sample preparation procedures followed different schedules depending on the type of formulation. A brief overview of the procedures are shown in Electronic Supplementary Information Figure ESI-1 and described in depth in paragraphs *2.2.1-2.2.4*

2.2.1. Tablets (T)

A quantity of 10 tablets was powdered with a pestle and mortar to a homogeneous mixture. Powder equivalent to 10 mg active compound (according to label claim) was transferred to a 10 mL volumetric flask and re-dissolved in 3 mL mobile phase mixture (A:B, 10:90, v/v) thereafter sonicated for 5-10 minutes and made up to the 10 mL mark with methanol:water (80:20, v/v). An Aliquot of the solution was filtered using 0.20 μm cellulose acetate filters (Sartorius, Germany) and the filtrate was used in preparing a working concentration that was subjected to LC-MS/MS analysis.

2.2.2. Capsules (C)

A quantity of 10 capsules was weighed with shells and granules. Thereafter, each of the 10 capsules was carefully opened and the content completely emptied into a mortar, and the granules, finely homogenized. Afterwards, the 10 empty shells were weighed collectively and the average weight of the granules determined by subtracting the weight of shells from the total weight of capsules and granules. Powder equivalent to 10 mg active compound (according to label claim) was transferred into a 10 mL volumetric flask and re-dissolved in 3 mL mobile phase A:B (10:90, v/v), thereafter sonicated for 5-10 minutes and made up to the 10 mL mark with methanol:water (80:20, v/v). The rest of the procedure was as already described in sub-section *2.2.1.*

2.2.3. Intravenous injection solutions (IV), ear and eye droplets (D)

Intravenous injection solution and ear and eye droplets were handled using identical procedures. The samples were hand shaken vigorously for a minute in their original packages. Thereafter a sample equivalent to 10 mg active compound (according to label claim) was transferred to a 10 mL volumetric flask and sonicated 5-10 minutes with 3 mL mobile phase A:B (10:90, v/v), and finally made up to the 10 mL mark with methanol:water (80:20,v/v). Gentamicin samples were prepared as above with the addition of 10 μL (10 mM trifluoroacetic acid) to the final working concentration. The rest of the procedure was as already described in sub-section *2.2.1.*

2.2.4. Suspensions (S)

Gels, suspensions and syrups were hand shaken vigorously for a minute in their original packages. Thereafter a sample equivalent of 10 mg active compound (according to label claim) by volume was transferred to a 10 mL volumetric flask and sonicated for 5-10 minutes with 3 mL mobile phase A:B (10:90, v/v) and finally made up to the 10 mL mark with methanol:water (80:20, v/v). The rest of the procedure was as already described in sub-section *2.2.1*.

2.3. LC-MS/MS analysis

LC-MS/MS analyses were performed on an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) hyphenated to a Sciex API 3000 triple-quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA). Collection and treatment of data were performed using Analyst software 1.4 (Applied Biosystems). The HPLC system consisted of a binary pump, a degasser, a cooled autosampler (4 ºC), an injector (10 µL) and a column oven (30 ºC). The column stationary phase was a Waters XTerra MS C_{18} (2.1 x 100 mm; 2.5 µm) and an equivalent 10 mm guard column. Flow rate was 250 µL/min and injection volume was 5 µL. The developed LC method consists of a gradient of the mobile phases A and B, starting with 1% (B) holding for 5 minutes (0-5 min) and increasing to 75% (B) during 15 minutes (5-20 min). Isocratic elution at 75% (B) followed for another minute (20-21 min). Mobile phase A was increased back to 99% within 1 minute (21-22 min) and equilibrated for another 8 minutes (22-30 min).

The MS was equipped with an electrospray ionisation (ESI) source (Turbo Ion Spray) operated in switching ionisation mode (negative and positive). The MS optimised source parameters were as follows: ion spray voltage: -3500V and 3500V and temperature: 400 ºC. Nitrogen was applied as nebulizer gas (10 L/min), curtain gas (6 L/min) and collision gas (10 L/min). MS detection was performed using multiple reaction monitoring mode (MRM). The precursor and product ions, and optimised compound specific parameters are given in Table 2.

2.3.1. LC-MS/MS analysis for Gentamicin

All procedures and parameters described in section *2.3.* applies to the analysis for Gentamicin with the exception of the composition of mobile phase A. In the analysis of Gentamicin, mobile phase A consisted of water:acetonitrile:methanol (90:6:4, v/v/v) only with 300 μL formic acid (98%) added per litre.

2.4. Validation

For the validation of the analytical procedure, the European Medicines Agency guideline [31] was followed. Calibration curves were prepared as neat standard dilutions from a 10 ppm mixed solution of all antibiotics. The calibration concentration range (in HPLC-vial) was 50-2000 ng/mL for the majority of the antibiotics, except for ciprofloxacin, trimethoprim and ceftriaxone where the calibration concentration range was 20-200 μg/mL. Instrument limit of detection (LOD) was determined from the calibration curves as 3 times the residual standard deviation of the linear regression divided by the slope. Furthermore, limit of quantitation (LOQ) was determined as 10 times the same ratio. The instrumental precision was investigated using neat standards at 200 and 400 ng/mL concentrations for a majority of the compounds, while 20 and 40 μg/mL was used for ciprofloxacin, trimethoprim and ceftriaxone.

Tablet and capsule method accuracy was studied using tablets from one location containing metronidazole with a label claim of 200 mg/tablet. Tablets were prepared according to section *2.2.1* and immediately after the filtration step; the sample solution was divided into two aliquots. The first aliquot was used as a blank (background concentration of metronidazole) while the second aliquot was spiked with 4 μg of all antibiotics available in tablets and capsule matrices (Table 1, T, C). By comparing the spiked pool with nominal concentration of 400 ng/mL the method accuracy was obtained. The concentration of the spiked pool for analytes, except that in the first aliquot used as blank was, compared with nominal concentration. For the analyte in the first aliquot used as

blank, the background concentration was subtracted from the spiked pool concentration for metronidazole, and then the resulting concentration compared with nominal concentration.

Intravenous injection, ear and eye drops method accuracy was assessed using an intravenous injection of penicillin G with a label claim of 300 mg/mL. Using the procedure from section *2.2.3* and just prior to the final dilution, the sample was divided into two aliquots. The first aliquot was used as a blank (background concentration of penicillin G) and the second aliquot was spiked with 4 μg of all antibiotics available in intravenous and ear and eye drops matrices (Table 1, IV, D). By comparing the spiked pool with the nominal concentration of 400 ng/mL the method accuracy was obtained. The method accuracy for the intravenous injection of gentamicin products was assessed using an intravenous injection of gentamicin with a label claim of 80 mg/mL. Using the procedure from section *2.2.4* and just prior to the final dilution, the sample was divided into two aliquots. The first aliquot was used as a blank (background concentration of gentamicin) and the second aliquot was spiked with 4 μg gentamicin. By comparing the spiked pool with nominal concentration of 200 μg/mL, the method accuracy was obtained.

Suspensions method accuracy was assessed using oral suspension of amoxicillin with a label claim of 25 mg/mL. Using the procedure from section *2.2.4* and just prior to the final dilution, the sample was divided into two aliquots. The first aliquot was used as a blank (background concentration of amoxicillin) and the second aliquot was spiked with 4 μg of all antibiotics used in suspensions (Table 1, S). By comparing the spiked pool with the nominal concentration of 400 ng/mL the method accuracy was obtained.

3. Results and discussion

3.1. LC-MS/MS method development and optimisation

The LC-MS/MS method was developed and validated for quality monitoring of antibiotics with the capacity to quantify individually as well as simultaneously 12 antibiotic agents as pure standards and drug product samples in various matrices. We observed low signal intensity for gentamicin when using the mobile phase A composition as

for the other 12 antibiotics. The signal intensity was improved when omitting triethylamine and ammonium formate. Consequently, gentamicin was analysed in a separate injection using mobile phase A only containing formic acid. As evidenced in Table 1, the physico-chemical properties of the compounds analysed show great variations in chemical structure, acid-base properties and solubility. However, all the compounds are fairly polar and make it possible to consider liquid chromatography in the reverse phase mode for method development. In addition, the binary gradient elution aided in handling the large diversity in acid-base properties and water solubility with respect to separation and resolution of the compounds. A representative chromatogram is shown in Figure 1. Co-eluting known compounds could still be identified and quantified as individual compounds because of the hyphenated MS/MS detector. Optimisation of compound specific parameters was performed on all thirteen compounds (Table 2). Precursor ions, mostly $[M-H]^-$ ions and $[M+H]^+$ ions, and fragment ions of the individual compounds were obtained for all compounds. Furthermore, fragment ions from the investigated antibiotics were verified by assessing the molecular structure. It was observed that ciprofloxacin, trimethoprim and ceftriaxone all had very good signal-to-noise intensities. A reason for ceftriaxone having better sensitivity compared to other included ß-lactam antibiotics (cefuroxime, ampicillin and penicillin G) could be that it is analysed in negative ionisation mode, whereas the three latter are analysed as positive ions (Table 2).

Various mobile phase compositions were investigated with varying amounts of additives for both the positive and negative ESI modes in order to obtain a sensitive and efficient HPLC separation of all compounds with good resolution. Varying concentrations of methanol, acetonitrile, formic acid, ammonium formate and triethylamine were investigated and a composition of: mobile phase A: water:acetonitrile:methanol (90:6:4, v/v/v) and mobile phase B: water:acetonitrile:methanol (5:65:30, v/v/v) for the gradient elution was found appropriate for the 12 simultaneously determined antibiotics, complemented by a modified mobile phase A for gentamicin as described under *2.3.1.* Analysis of gentamicin required a separate injection (chromatogram not shown). The efficiency of the chromatographic separation is shown in Figure 1. Even though amoxicillin and

clavulanic acid, co-eluted (Figure 1) the different MRM ion transitions allowed for separation and identification of the two compounds, respectively.

3.2. Optimization of sample preparation procedure

The challenge of sample preparation involving 13 compounds with varying physico-chemical properties especially log P values, had to be tackled to enable a homogenous mixture of all compounds to be obtained. Methanol and acetonitrile were initially investigated as a solvent for dissolving all analytes. However, mobile phase A:B (10:90, v/v) together with methanol:water (20: 80, v/v) in a volume ratio of 3:7 provided a clear solution (data not shown). Sonication of solutions of compounds, both in the pure reference standard form or in dosage forms played a key role in the sample preparation step. It was observed that some compounds (trimethoprim, metronidazole, sulphamethoxazole and amoxicillin) crystallised on the inner surfaces of storage containers from solution when sonication time was between 2-5 minutes. However, when the compounds were sonicated for 5-10 minutes this was not observed (data not shown).

3.3. Validation of methodology

Calibration curves for all 13 analytes were obtained and good linear dynamic range (50-2000 ng/mL) was observed for all analytes, except for ciprofloxacin, ceftriaxone and trimethoprim that were evaluated in the concentration interval 5-200 μg/mL, and gentamicin in 100-400 μg/mL (Table 3). Coefficient of determination (r^2) were greater than 0.9847 for all compounds, and instrument LOD for all analytes were in the range of 55 ng/mL to 58 μg/mL and LOQ in the range of 185 ng/mL to 194 μg/mL (Table 3). Instrument precision (or repeatability) was investigated using six injections of standard solution at two different concentration levels (200 and 400 ng/mL, Table 3). Precision expressed as per cent coefficient of variation (CV) at two concentration levels was between 1-12%. These results indicate a good method precision with the highest variability obtained at the lowest tested concentration level.

High mean recoveries with very low variations were obtained for all compounds from the accuracy study (Table 3). For tablets (T) and capsules mean recoveries were between 97-101%, however a value of 125% was obtained for ampicillin (AMP) likely due to matrix effects. Mean recoveries were 96-100% for suspensions and syrups (S) and 97-101% for injectables (IV). Based on these data, the developed methodology was applied to a number of samples gathered in Ghana to enable us to quantify 13 antibiotics in pharmaceutical formulations. The method was able to detect substandard and quality antibiotics within the health sector of Ghana.

3.4. Application

The method was applied to the assay of pharmaceutical products sampled from Ghana and a few (amoxicillin, metronidazole, erythromycin, tetracycline and sulphamethoxazole and trimethoprim products; all tablets) sourced from the United Kingdom. Besides the discussed validation in section 3.3, the UK samples were also used as a mean to further validate the developed method, as any drug on the EU marked is under strict quality assessment control. The samples analysed (six different tablets ranging from 80 to 400 mg/tablet) from UK all had percentage content of more than 98% of the label guarantee (Figure 2).

The method thus, could be employed in detecting substandard or counterfeit antimicrobial products used as essential medicines in countries like Ghana where all the agents studied are listed. Such information and data could help in the global fight against antimicrobial resistance. It is evident from Figure 2, the LC-MS/MS method developed was successfully employed to determine antibiotic drug formulations that are of good quality, and contained the right amounts of active ingredient and others that are of poor quality (substandard) containing low amounts of active ingredients as their respective label claims and with amounts far lower than their label guarantee. In the sampled tablets and capsules some antibiotics were found as low as 41% of label guarantee (ETM), and only four of the ten tested were above 90% of label guarantee (AMX, CLV, AMP, TMP, Figure 2). In the sampled suspensions and syrups, merely three antibiotics (STX, CPF and ETM) were above 90%, and others were as low as 18% (TMP, Figure 2). Quality variations among formulations gave an indication that

intravenous products have a higher quality than suspensions and syrups and a much higher quality than capsules and tablets. Six of ten antibiotics were above the 90% label claim, and e.g. TCC was as low as 53% (Figure 2). Variations with respect to the amount of active ingredient determined, among formulations, source of product (from regulated health facilities or sources to un-regulated facilities or sources) were observed (data not shown). Antibiotic drug formulations with mean percentage content (as per label guarantee or claim) below pharmacopoeia specifications (usually less than 90% of label guarantee) [32] could result in sub-maximal therapeutic effects and related health consequences such as high cost of healthcare. This could result in loss of confidence in the healthcare system and ultimate public health concerns.

4. Conclusions

A method was described for the quantification of 13 antibiotics in pharmaceutical dosage forms using LC-MS/MS. The developed method showed high sensitivity, accuracy and precision and was employed in analysing several commercially available antibiotic drug products available on the Ghanaian market. The procedure could find use in the detection of substandard or counterfeit antimicrobial products and trace quantities of these antimicrobial agents in pharmaceutical products either as APIs or adulterants or cross contaminants. Several of the investigated drug products (more than 65% of sampled antibiotic products) showed levels of antibiotics below 90% of label claim which is an indication of the availability of substandard antibiotic drug products in both the formal and informal health sectors. This could have public health consequences on the quality of healthcare delivery in Ghana.

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http://admerproject.org/). Special thanks go to the ADMER project contributors in Ghana and Denmark, and The Ministry of Health of Ghana.

References

1 H.V.Hogerzeil, M.J. de Goeje, I.O.Abu-Reid. Lancet, 338, (1991), 754–5.

2 R. B.Taylor.,OShakoor,R. H.Behrens, Lancet, 356, (1995), 122.

3 O.Shakoor, R. B.Taylor, R. H Behrens, Tropical Medicine and International Health, 2, (1997), 839–845.

4 WHO. Counterfeit and Sub-standard Drugs in Myanmar and Vietnam. Geneva: WHO, (1999), 1–55.

5 B.Pecoul, P.Chirac, P.Trouiller et al., JAMA, 281, (1999), 361–7.

6 D.B.Menkes, BMJ, 315, (1997), 1557–8.

7 J.Roy, World Health Forum, 15, (1994), 406–7.

8 M.Hanif, M.R.Mobarak, A.Ronan et al., BMJ, 311, (1995), 88–91.

9 Wan Po AL. Lancet, 357, (2001), 1904.

10 P.Newton, S.Proux, M.Green et al., Lancet, 357, (2001), 1948–50.

11 C. Baxerres, Jean-Yves Le Hesran, Benin,J.Soc. Science & Med., 73, (2011), 1249-1256.

12 J.V.Videau,B. Fundafunda, WHO Drug Information (2000), 77–81.

13 WHO. Quality assurance of pharmaceuticals. Vol 1. Geneva: World Health Organization, 1997.

14 WHO. Quality assurance of pharmaceuticals. Vol 2. Geneva: World Health Organization, 1999.

15 WHO. www.who.int/medicines/services/counterfeit/faqs/15/en/ (March, 2013)

16 Bull World Health Organ, 71, (1993), 464–70.

17 Lancet, 350, (1997), 601.

18 S. Afu Trop Med Int Health 4, (1999) 73.

19 R. Verduin-Muttiganzi, G.Verduin-Muttiganzi, Trop Med Int Health 3, (1998), 602.

20 S.Pincock, BMJ, 327, (2003), 1126.

21 L.Gibson, BMJ, 328,(2004), 486.

22 P.M.Rudolf, I.B.Bernstein, N Engl J Med, 350, (2004), 1384–6.

23 WHO report; Study on the Quality of Antibiotics in Ghana, 2010.

24 WHO. Counterfeit Drugs: Guidelines for the Development of Measures to Combat Counterfeit Drugs. Geneva, (1999), 1–60.

25 R. H.Behrens, A. I.Awad, R. B.Taylor, Tropical Doct. 32, (2002), 1-2.

26 S. Gallien,E. Duriez, K. Demeure, B. J Proteomics. 81, 2013,148-58

27 Liberato Brum Junior , Flávia de Toni Uchoa ,Sílvia Stanisçuaski Guterres, Teresa Dalla Costa, J. Liq. Chrom.& Rel. Tech. 1, 32, (2009), 2699–2711

28 E.Pérez-Carrera, M.Hansen, V.M.León, E.Björklund, K.A.Krogh, B.Halling-Sørensen, E.González-Mazo, J.Anal. and Bioanal. Chemistry, 398, 3, (2010), 1173-1184.

29 Xesús Feása, Lei Yeb, Seyed V. Hosseinic, Cristina A. Fentea, Alberto Cepedaa, J. of Pharm.and Biomed. Anal. 50, 5, (2009), 1044–1049.

30 C.Barton, P.Beck, R.Kay, P.Teale, J.Roberts, J.Proteomics. 9 (11), (2009), 3058-65.

31 International Conference on Harmonization (ICH), Validation of analytical procedures: text and methodology, ICH Q2 (R1), 1995.

32 United States Pharmacopoeia XXVII, General official monographs for USP 27, The United States Pharmacopoeia Inc., Rockville, MD, 2004, 139-1750.

33 David B. Troy, Joseph Prince Remington, Paul Beringer, Remington's: The Science and Practice of Pharmacy. 21st Edition, (2006), 1548.

34 A. C Moffat, M.D Osselton , B.Widdop, Clarke's Analysis of Drugs and Poisons, 3rd Edition, (2004) CD-ROM. London: Pharmaceutical Press.

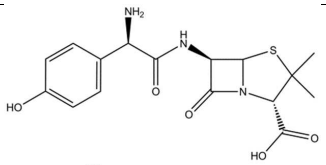
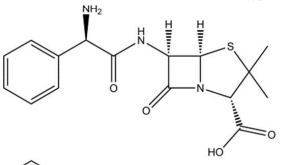
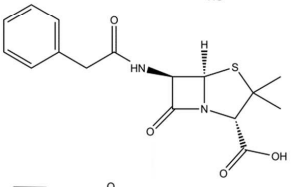
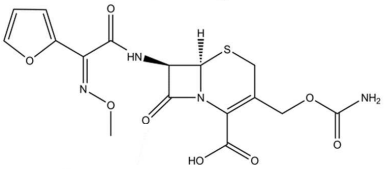
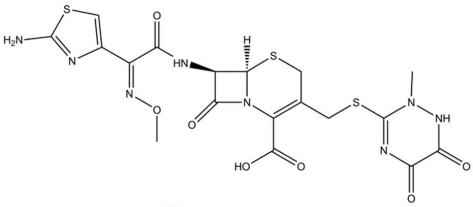
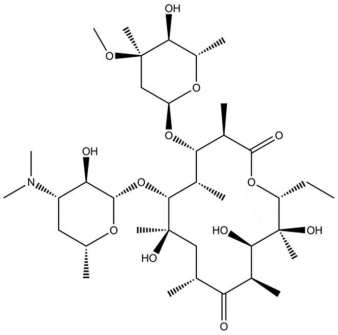
Figure Captions

Figure 1. Chromatogram of neat standard solution containing 12 of the 13 investigated antibiotics in positive and negative ESI modes. Peak numbers (signal multiplied with factor); 1, clavulanic acid (x10); 2, amoxicillin (x10); 3, metronidazole; 4, ampicillin (x3); 5, ceftriaxone (x50); 6, trimethoprim (x10); 7, cefuroxime (x10); 8, tetracycline (x2, off-set: -1.25 min and +25 000 cps); 9, ciprofloxacin (x10); 10, sulphamethoxazole; 11, d_4-sulphamethoxazole (internal standard); 12, penicillin G (x0.5); 13, erythromycin (x0.2). Gentamicin is analysed in a separate injection (chromatogram not shown).

Figure 2. Graph showing 13 antibiotics analysed in various formulations as their mean % content of their respective label guarantee. Top; Six different tablets from UK analysed with the developed methodology (label claims per tablet; AMX 250 mg, MTX 400 mg, ETM 250 mg, STX 400 mg, TMP 80 mg and TCC 250 mg, all n=3). Three lower boxes are analysis results of samples from Ghana (no replicates).

TABLES

Table 1: Name, CAS number, chemical structures and physicochemical properties of the investigated antibiotics Physicochemical properties include molecular weight (Mw), acidity constant (pK_a), water solubility (S_w), octanol-water partition coefficient (log P).

Name	Product	Chemical structure	Mw (g/mol)	pK_a	S_w (g/L)	
Amoxicillin (AMX) [26787-78-0] ~~T,C,S,IV~~	T,C,S,IV		365.4	2.4,7.4,9.6[c]	3.43[a]	0.87[a]
Ampicillin (AMP) [69-53-4] ~~C,S,IV~~	C,S,IV		349.4	2.5,7.3[c]	10.1[a]	1.35[a]
Penicillin G (PNG) [61-33-6] ~~IV~~	IV		334.4	2.74[a]	0.21[a]	1.83[a]
Cefuroxime (CFX) [55268-75-2] ~~T,S,IV~~	T,S,IV		424.4	1.8,2.7,4.1[b]	0.15[a]	-0.16[a]
Ceftriaxone (CTX) [73384-59-5] ~~IV~~	IV		554.6	3,3.2,4.1[c]	-	-
Erythromycin (ETM) [114-07-8] ~~T, S~~	T, S		733.9	8.88[a]	0.0014[a]	3.06[a]

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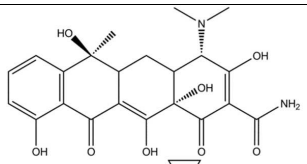
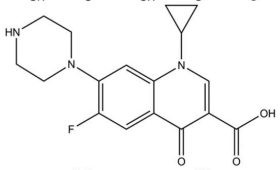
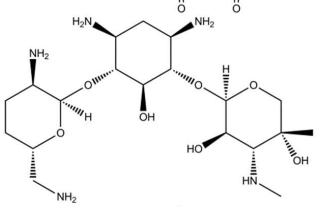
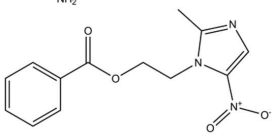
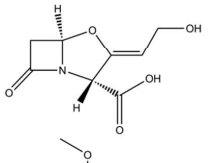
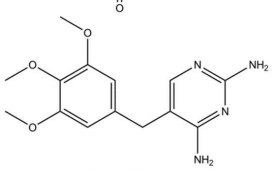
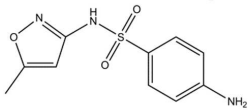
(Table 1 continued)

Name	Product	Chemical structure	Mw (g/mol)	pK_a	S_w (g/L)	
Tetracycline (TCC) [60-54-8] ~~C~~	C		444.4	3.3[a]	0.23[a]	-1.30[a]
Ciprofloxacin (CPF) [85721-33-1] ~~T, S, IV, D~~	T, S, IV, D		331.3	6.09[a]	30.0[a]	0.28[a]
Gentamicin (GMC) [1403-66-3] ~~IV, D~~	IV, D		477.6	-	-	-1.88[a]
Metronidazole (MTZ) [443-48-1] ~~T, S, IV~~	T, S, IV		171.2	2.5[c]	9.50[a]	-0.02[a]
Clavulanic acid (CLV) [58001-44-8] ~~T, S, IV~~	T, S, IV		199.2	-	-	-2.04[a]
Trimethoprim (TMP) [738-70-5] ~~T, S~~	T, S		290.3	7.12[a]	0.40[a]	0.91[a]
Sulphamethoxazole (STX) [723-46-6] ~~T, S~~	T, S		253.3	5.6[c]	0.61[a]	0.89[a]

[a]ChemIDplus (US National Library of Medicine).[b]Remington's: The Science and Practice of Pharmacy[33].[c]Clarke's analysis of drugs and poisons[34]. Active compound in tablets (T), capsules (C), suspensions and syrups (S), intravenous injections (IV), ear and eye droplets (D).

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Table 2: Mass spectrometry specific parameters in the validated LC-MS/MS analytical method indicating ion transitions for thirteen investigated antibiotics.

Compound	Retention time (min)	Mode of Ionisation	Precursor ion>quantifier	Dwell time (ms)	Declustering Potential (V)	Focusing Potential (V)	Entrance Potential (V)	Collision energy (eV)	Collision cell exit potential (V)
First period									
Amoxicillin	1.22	Negative	364.3>222.8	500	-50	-200	-10	-20	-10
Clavulanic acid	1.51	Negative	197.9>135.7	500	-30	-100	-5	-10	-20
Second Period									
Metronidazole	3.01	Positive	172.1>128.0	200	20	50	10	20	15
Ampicillin	3.51	Negative	348.1>206.8	200	-20	-100	-15	-20	-10
Third period									
Ceftriaxone	5.77	Positive	555.4>396.1	1000	20	150	15	20	30
Trimethoprim	6.56	Positive	292.2>262.1	1000	30	150	10	30	10
Fourth period									
Cefuroxime	9.28	Negative	423.0>317.8	2000	-20	-100	-10	-10	-15
Fifth period									
Tetracycline	12.10	Negative	443.0>357.9	200	-20	-200	-10	-30	-10
Ciprofloxacin	12.23	Positive	332.6>245.0	100	50	200	10	30	20
Sixth Period									
Sulphamethoxazole	13.39	Negative	252.0>155.7	500	-20	-100	-5	-20	-15
Seventh period									
Penicillin G	15.79	Negative	333.1>191.8	500	-30	-100	-10	-10	-10
Eighth period									
Erythromycin	17.63	Positive	734.2>158.3	200	20	100	10	35	30
GMC method									
Gentamicin	1.2	Positive	464.0>322.3	200	30	200	10	30	10

Table 3: Validation parameters summarised for thirteen investigated antibiotic compounds. Linear dynamic range (LDR), coefficient of determination ~~correlation coefficient~~ (r^2), instrument LOD and LOQ were determined from linear regression curves. Concentration units of parameters investigated in ng/ml except ciprofloxacin, ceftriaxone, trimethoprim and gentamicin whose units are in µg/ml.

		AMX	CLV	MTZ	AMP	CFX	TCC	CTX	STX	TMP	CPF	PNG	ETM	GMC
LDR		50-2000	50-2000	50-2000	50-2000	50-2000	50-2000	5-200	50-2000	5-200	5-200	50-2000	50-2000	100-400
	r^2	0.9958	0.9937	0.~~999~~999888	0.~~999~~999863	0.9913	0.9720	0.~~989~~989783	0.9807	0.9964	0.9749	0.9948	0.~~8829~~98829	0.~~989~~989747
LOD		55.5	68.3	91.3	101.2	80.4	145.9	12.8	120.6	5.2	13.8	62.4	94.0	58.3
LOQ		184.9	227.6	304.2	337.4	268.0	486.2	42.6	401.9	17.2	45.9	207.9	313.4	194.2
Precision (CV)	200 ng/mL	2.5	7.9	6.0	6.6	7.8	6.9	11.7	3.8	4.9	11.1	5.6	5.0	2.9
	400 ng/mL	2.3	3.5	3.7	1.9	3.8	8.9	5.5	0.8	8.3	11.2	1.9	3.7	2.5
Recovery (±CV)	T/C	99±2	98±8	101±3	125±1	100±6	98±1	-	100±1	100±1	97±5	-	100±1	-
	S	99±2	98±8	99±3	96±6	99±9	-	-	100±2	100±2	97±9	-	98±11	-
	IV	98±8	101±3	99±2	100±2	98±6	-	97±7	-	-	99±9	98±0.3	-	98±2

- Not available in the dosage form

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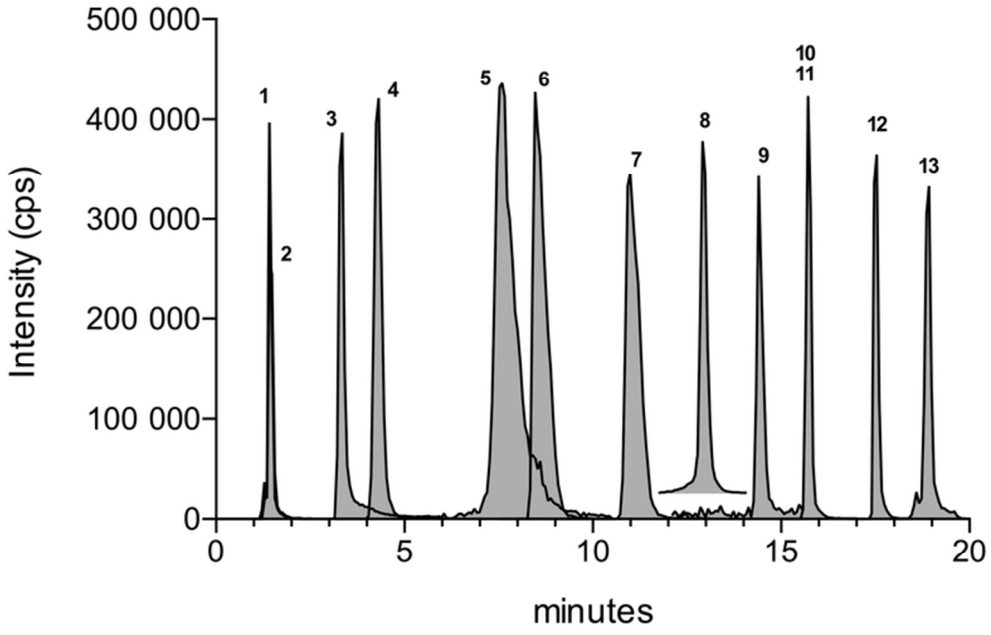

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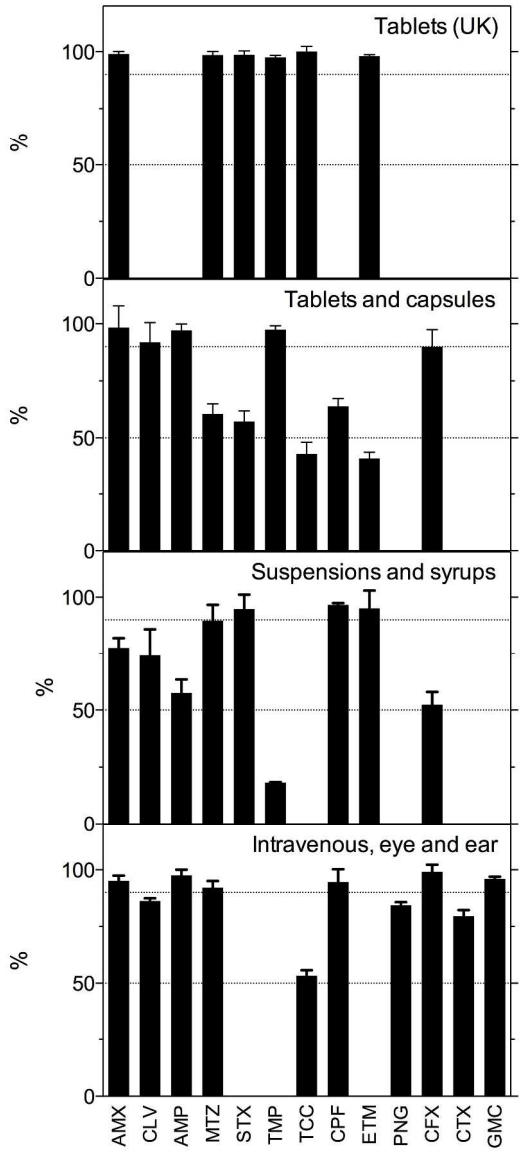

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