Analytical Methods

Detection of Degraded, Adulterated, and Falsified Ceftriaxone Using Paper Analytical Devices

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Complete List of Authors:	Bliese, Sarah; University of Notre Dame, Chemistry and Biochemistry Maina, Mercy; Moi Teaching and Referral Hospital, Pharmacy Were, Phelix; AMPATH Kenya, Academic Model Providing Access to Healthcare Lieberman, Marya; University of Notre Dame, Chemistry and Biochemistry

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PAPER

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Sarah L. Bliese[a], Mercy Maina,[b] Phelix Were,[b] and Marya Lieberman[a,*]

Substandard or falsified versions of ceftriaxone have been found in the supply chains of many countries. Ceftriaxone is an injectable antibiotic often used for treatment when resistance to other antibiotics has developed, so detection of bad quality formulations is of keen interest to the global health community. We found that a paper analytical device (PAD) can detect and differentiate degraded, adulterated, and falsified ceftraixone injectable formulations. PAD analysis of eighty blinded formulations prepared in lab (20 falsified, 20 thermally degraded, 20 adulterated, and 20 good quality) had a 95% accuracy in classifying the formulations. Forty dosage forms collected in western Kenya were also assessed, and the PADs correctly identified three of four substandard samples. The sample that was missed had an 87.1% ceftriaxone content, which is just outside the 90-120% pharmacopeia range. The deterioration observed during storage of ceftriaxone solutions at room temperature shows a 2-3 day induction period, followed by rapid auto-catalyzed base degradation that quickly destroys the ceftriaxone. The instability of this drug highlights the need for inexpensive point-of-care testing to monitor ceftriaxone quality.

1. Introduction

Ceftriaxone is a broad-spectrum injectable antibiotic on the WHO Model List of Essential Medicines. (Organization, WHO Model List of Essential Medicines (19th List), 2015) Solutions of ceftriaxone are known to undergo rapid degradation (De Diego, Godoy, & Mnnickent, 2010), so the injectable drug is typically packaged as a solid for reconstitution with sterile water. As a third-generation cephalosporin antibiotic, ceftriaxone is often used for treatment when resistance to other antibiotics has developed, and it is the standard of care in some countries for patients with life-threatening infections such as bacterial sepsis. (WHO, Republic of Kenya Clinical Management and Referral Guidelines, 2009) A recent case report highlights the clinical importance of ceftriaxone quality (Nickerson et al, 2016):

> In February 2013, a case of bacterial meningitis following a middle ear infection was diagnosed in an adolescent at the Mulago National Referral Hospital in Kampala, Uganda. Once-daily treatment with 2 g of intravenous ceftriaxone administered according to guidelines failed, and the patient died. To determine whether the patient's treatment failure and subsequent death might be related to the ceftriaxone product administered, a sealed vial similar to the one administered to the patient was analyzed at the University of Ottawa, Canada, and was found to contain only 0.455 g of the drug, not 1 g as stated by the manufacturer.

This case shows how important is the role of the drug regulatory agency (DRA) in the health care system. According to a WHO review, 1 in 10 products in low- and middle-income countries (LMICs) are substandard or falsified. (WHO, 2017) Analytical methods that can speed up or scale up the detection of these bad quality pharmaceuticals are of interest to the global health community. (Newton et al, 2010 and Kovacs et al, 2014) If a product is found to be underdosed, more analytical information is needed: is the product under-dosed due to adulteration, or did significant decomposition of the active pharmaceutical ingredient (API) occur? The first problem signals a failure of good manufacturing practice (GMP) while the second could result either from a GMP failure or from problems further down the supply chain, such as poor storage conditions or improper dispensing practices. Ceftriaxone is at particular risk because of its known thermal instability, clinical utility, and the past examples of problem products. (Obaid, 2009)

Pharmacopeia assays for ceftriaxone use high performance liquid chromatography (HPLC) to assay the API. The capital cost, operating costs, and need for trained operators and other technological infrastructure associated with these analytical instruments make them a scarce resource for LMICs. HPLC can identify some degradation products, but those tests are not often carried out in LMICs due to the cost of the necessary reference materials and lack of capacity for HPLC-MS. Most field screening tests can only detect products that are fake or grossly substandard (<50% API content) and cannot tell why a drug is substandard. The GPHF Minilab, a semi-quantitative

[a] University of Notre Dame, Department of Chemistry and Biochemistry Notre Dame, IN 46556, USA. Email: mlieberm@nd.edu
[b] Moi Teaching and Referral Hospital, Dept. of Pharmacy, Eldoret, Kenya
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thin-layer chromatography (TLC)-based test that is one of the most common field screening tools for pharmaceuticals in LMICs, (GPHF, 2017) is designed to detect API concentrations below 80%, (Janke, 2001) rather than below the 90% level that marks substandard products in most pharmacopeia monographs. Reliable detection of products with API content <80% requires more than one week of training, (Risha et al, 2006). This is because TLC outcomes vary depending on the user's skill and ability to identify the location and size of chromatographic spots. (Pan & Ba-Thein, 2018).

We developed a paper analytical device (PAD) for analysis of solid dosage forms. (Weaver et al, 2013) The PAD gives accurate classification of good quality and falsified tablets and capsules. However, it is difficult to apply a controlled quantity of the solid sample to the paper, which hinders detection of substandard products. (Weaver et al, 2015) Here we show that when the PAD is dosed with aliquots of a liquid dosage form and the colors are analyzed by principal component analysis (PCA), the PAD accurately classifies ceftriaxone dosage forms as good quality, degraded, adulterated, or falsified.

2. Experimental

2.1 Pharmaceutical samples

Genuine samples of injectable ceftriaxone were obtained from western Kenya in 2016-2017 by covert shoppers, who recorded manufacturer, lot number, and expiration date. Ethical approval for the collection of the samples is necessary due to the element of deception involved in covert shopping and the need to send samples across national boundaries for assay. Permission was obtained from the Moi Teaching and Referral Hospital IREC (protocol #000836). Upon arrival at UND the samples were placed into cold storage at 4°C. The vial of ceftriaxone was weighed prior to opening and then after removal of powder to get total dosage mass. Portions of powder were removed for preparation at a nominal concentration of 110 mg/mL for PAD testing and diluted to a nominal concentration of 0.25 mg/mL for HPLC analysis as described in section 2.3. As described in our research protocol, the Kenyan Pharmacy and Poisons Board was regularly briefed on the PAD project and kept informed of all assay results.

2.2 Fabrication of the paper analytical device

These PADs are based on Weaver et al. 2013, with several modifications to the library of chemical tests described in detail in the supporting information (SI Figure 1). The following materials were used for the colorimetric tests on the paper analytical device: $NiCl_2(H_2O)_6$ and $FeCl_3(H_2O)_6$ were purchased from Fischer Scientific. Dimethylglyoxime, ninhydrin, sodium potassium tartrate, p-toluenesulfonic acid, and povidone (362,000 average molecular weight) were purchased from Alfa Aesar. Potassium carbonate, potassium iodide, and acetonitrile were purchased from VWR. $CuSO_4(H_2O)_5$, sodium hydroxide, $Co(NO_3)_2(H_2O)_6$, iodine, $Na_2Fe(CN)_5NO$, and p-nitroaniline were purchased from Sigma Aldrich. Potassium thiocyanate was purchased from Honeywell. Tris base and sodium nitrite were purchased from J.T. Baker. Sodium 1,2-napthoquinone-4-sulfonic acid was purchased from Acros Organics. The paper was Ahlstrom 319 (Midland Scientific, Chicago IL).

Using the Adobe Illustrator files provided in the Supporting Information, the QR code, fiducials, and lane markers were printed using a laser printer. The front and back wax lane markers and a set of wax fiducials were then printed using a Xerox ColorQube 8570N printer. The PADs were baked in a drying oven with paper towels shielding the metal racks for 7 minutes at 100°C to allow the wax to reflow. To check the sealing of the front and back wax, several lanes on each page were tested with drops of DI water to see if the water could no longer flow into adjacent lanes.

Reagents were placed into two 96-well plates as shown in SI Figure 1, and a spoke inoculating manifold (8 x 6 array, Dan-Kar Corp.) was dipped into each plate and stamped onto the blank PADs to transfer small droplets of each reagent to the appropriate lane positions. This stamping process transfers approximately 2 µL at each spotting site. One plate transfers reagents to lanes A, C, E, G, I, and K; the other to lanes B, D, F, H, J, and L. See supporting information for a review of the chemistry responsible for each lane's color results. To determine the stability of the PADs, two sets of PADs were stored in heat-sealed foil packs (Associated Bag) at room temperature for 2-4 weeks.

2.3 HPLC assay of ceftriaxone

System suitability testing was performed following United States Pharmacopeia (USP) <1225> and USP <1226>; briefly, this involves measuring analytical metrics for the HPLC chromatogram, establishing the linear range, testing accuracy and precision, and performing a matrix spike-recovery experiment to assess sample preparation and resolution of degradation products from the ceftriaxone peak. The system suitability results are summarized in SI (Table S1).

HPLC assays were performed using a Waters e2695 High Performance Liquid Chromatograph with a Waters 2487 Dual-Wavelength Absorbance Detector set to 240 nm. The method was a modified version of the USP method. (United States Pharmacopeial Convention, 2016) The column was a Kintex 250 x 4.6 mm, C18 column with 5 µm particle size. Ceftriaxone disodium hemiheptahydrate (TCI, lot number QWKDF-OO, 99.9% purity) secondary standard was used as the reference material. Ceftriaxone solutions were prepared at a concentration of 0.25 mg/mL in Millipore water. The method was isocratic with a 10-minute run time at a flow rate of 1.0 mL/min. The injection volume was 20 µL. The mobile phase consisted of 3.2 g tetraheptylammonium bromide (Alfa Aesar), 4 mL pH 5.0 citric acid buffer (0.19 mM, Fischer Scientific), 44 mL pH 7.0 phosphate buffer (0.14 mM, Fisher Scientific), 400 mL HPLC grade acetonitrile (Sigma-Aldrich), and was brought to 1000 mL using Millipore water (Synergy UV). Ceftriaxone's retention time was 4 minutes, see SI section on system suitability for a sample chromatogram.

2.4 LC-MS analysis

To identify the degradation products observed on the HPLC, the injection volume was increased to 100 µL, which saturated the UV detector. As each peak emerged, an Eppendorf tube was used to collect the eluting fraction. The fractions were then directly aspirated into a Bruker micrOTOF-Q II mass spectrometer operated by the Mass Spectroscopy and

Proteomics Facility at the University of Notre Dame for identification.

2.5 Ceftriaxone training and testing sets

A training set of 20 good quality, 20 degraded, 20 adulterated, and 20 falsified ceftriaxone samples was generated from ceftriaxone disodium hemiheptahydrate (TCI, lot number QWKDF-OO, 99.9% purity) secondary standard. According to USP standards, "good quality" injectable ceftriaxone contains 90-120% API content. Degraded samples were prepared by allowing a 110 mg/ml solution of good quality ceftriaxone to stand at room temperature until the API content was between 50 and 70%, and at least one of the degradation products was detectable by HPLC. Adulterated samples were prepared by adding talc or starch in a 1:1 w/w ratio to good quality solid ceftriaxone. These samples were prepared at a nominal 110 mg/ml ceftriaxone concentration, but the actual ceftriaxone content was only 50% of that. Falsified samples were prepared by substituting amoxicillin, acetaminophen, or sodium chloride for ceftriaxone; after preparation of the nominal 110 mg/ml ceftriaxone solution, these samples contained 0% of the stated API.

The PAD images of the training set underwent principal component analysis (PCA) and threshold values for PCA 1 and PCA 2 were set to differentiate between good quality, degraded, adulterated, and falsified ceftriaxone. The PCA thresholds from the training set were applied to all the other samples in this paper.

An independent set of 20 good quality, 20 degraded, 20 adulterated, and 20 falsified ceftriaxone samples was generated from a good quality ceftriaxone dosage form (HPLC assay 98%) collected in Kenya. These samples were used to evaluate the accuracy of the PCA metrics.

Another 80 samples were generated from reference ceftriaxone to assess the accuracy of visual reads; this set consisted of 20 good quality standards, 20 thermally degraded (API content was between 70-80%), 10 adulterated (API content 50%) and 30 falsified. A novice categorized these 80 images as good or bad quality ceftriaxone based off a standard reading guide, and the PCA thresholds from the training set were applied to classify the samples.

Finally, the 40 samples of ceftriaxone injectable collected in Western Kenya (section 2.1) were evaluated using both PADs and HPLC.

2.6 PAD imaging and computer image processing

Mocked-up samples of good and bad quality injectable ceftriaxone were prepared as described in section 2.5 and blinded so the researcher running the PADs did not know the identities of the samples. Each sample was run on one PAD by preparing a solution with a nominal concentration of 110 mg/mL (the concentration of the injectable when reconstituted for injection), and pipetting 2 µL aliquots on each of the 12 lanes of the PAD at the position of the "swipe line" (see SI Figure 1). The 2 µL aliquots can be dispensed from an inexpensive volumetric capillary pipet or an automatic pipettor. The samples were allowed to dry for about 5 minutes and were then developed by placing the bottom of the PAD into a shallow dish of DI water until water reached the top of Lane A and the pink timer dot appeared. The PAD was laid on a paper towel to dry for 2-3 min.

The PAD was placed inside a light box and photographed with two mobile phone cameras: a Google Pixel and an LG Spectrum 2. The light box was made from a shoebox and measured 19.5 cm x 34 cm x 11.5 cm. The box contained a string of white light LEDs that were plugged into wall current (battery operated LEDs fade as the battery looses power). The LEDs were covered with plain writing paper to diffuse the light throughout the box. A hole was cut into the top to accommodate the cell phone camera. The lane width for images acquired in the lightbox was about 40 pixels.

ImageJ (Schneider et al, 2012) software was used to quantify the red, green, and blue (RGB) intensity of color spots appearing in each lane. The mean intensity was determined by first, inverting the image (so large numbers correspond to high color intensity), selecting a square of 40 by 40 pixels around the strongest part of the colored spot, and then collecting the mean RGB color values.

The software package R (Bivand et al, 2013) was used to run principal component analysis on the color intensity values collected by ImageJ.

3. Results

3.1 Using PADs to screen ceftriaxone

Solid ceftriaxone is provided in a septum-capped vial with a separate container of sterile water. The solid gives color reactions in lanes F, H, and K. Based on the presence of these colors, the PAD is able to detect the solid ceftriaxone dosage form (Figure 1A) and differentiate it from many other pharmaceuticals. In clinical use, the dosage form is reconstituted to 110 mg/mL for injection or 330 mg/mL for IV administration, so the tests in this study were run using a solution with the nominal concentration of 110 mg/mL ceftriaxone. Aliquots of 2 uL were pipetted onto each of the 12 lanes of the PAD, equivalent to a 0.22 mg dose of ceftriaxone in each lane. Good quality ceftriaxone (Figure 1B) is readily distinguishable from a solution of substitute APIs such as amoxicillin (Figure 1E). Adulterated samples (Figure 1D) give the same bar code, but lower color intensities, and as ceftriaxone degrades (Figure 1C), the green color in lane F darkens and a purple color appears in lane H. The question we needed to answer was whether these changes were reproducible enough to have predictive utility.

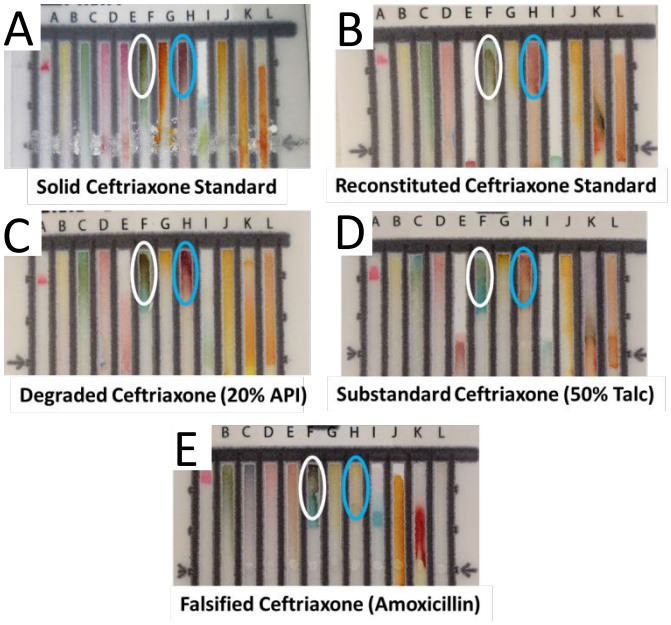

Figure 1. PAD Images for Ceftriaxone. A) solid ceftriaxone standard, 1 mg/lane B) 2 µL/lane of 110 mg/mL ceftriaxone standard, C) 2 µL/lane of degraded standard ceftriaxone (20 mg/mL ceftriaxone), D) 2 µL/lane of 50% w/w ceftriaxone-talc (55 mg/mL ceftriaxone), and E) 2 µL/lane of 110 mg/mL amoxicillin suspension.

3.2 Tracking the degradation of ceftriaxone.

According to the USP monograph for injectable ceftriaxone sodium, the API content must be in the range 90-120%. When a solution of 110 mg/L ceftriaxone stands at 23 °C, it undergoes hydrolysis and other degradation reactions, as shown in Figure 2.

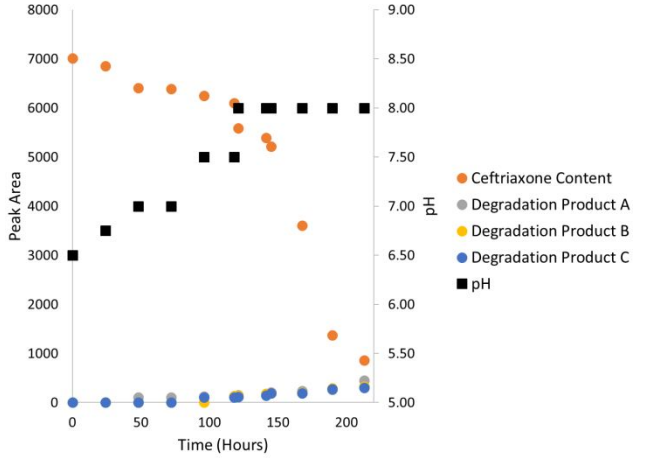

Figure 2. Degradation of ceftriaxone solution at 23˚C. The HPLC peak areas (circles) are plotted on the primary y-axis and the pH of the solution (squares) on the secondary y-axis.

The ceftriaxone concentration slowly drops after 24 hours, and by 100 hours, its level falls below 90%, making the injectable substandard even if it started out at full potency. Over the same time interval, the pH of the solution slowly rises from 6 to 7 and three degradation products are observed by HPLC (SI Figure 3). These degradation products are identical to products formed under basic hydrolysis conditions in the literature. (Zajac and Muszalska, 1998, SI Figure 4) Once the pH exceeds 7, the degradation rate of ceftriaxone increases sharply, and by day 9, ceftriaxone is no longer detectable.

The chemical changes that occur during the degradation of ceftriaxone cause characteristic color changes in lanes F and H on the PAD (SI Figure 5). The rise in pH is one of the chemical composition changes that the PAD detects. Other pharmaceuticals, such as amoxicillin-clavulanate, undergo autocatalyzed base degradation and are of interest for future quantitative study with the PAD. The RGB pixel intensities for six of the lanes (C, F, G, H, K, and L) were used as inputs for principal component analysis (PCA), which showed clear grouping of the good quality injectable vs various types of bad quality injectable ceftriaxone.

Figure 3 shows PCA results for the training set prepared from ceftriaxone standards, as well as a test set prepared from dosage forms. Principal component (PC) 1 explains 77% of the data variance, and consists of equal portions of the blue and green color channel intensities in lane F. An additional 11% of the data variance is accounted for by PC 2, which takes the blue and red color channel intensities in lane H into account. The average RSD for these four channel intensities (triplicate PADs, data shown in figure S5) was 6%. The good quality training samples were characterized by PC 1 values >0.04 and PC 2 values >0.06. The degraded training samples were characterized by PC 1 values >0.04 and PC 2 values <0.06. The adulterated training samples were characterized by PC 1 values <0.04 and PC 2 values <0.06, and the falsified training samples had PC 1 values <0.04 and PC 2 values >0.06. These threshold PC 1 and PC 2 values were used to classify the blinded test set as good quality, degraded, adulterated, or falsified. The specificity and sensitivity of the classifications on this independent data set were both 98%. One falsified sample that was made of cephalexin was classified as adulterated, although a human reader could detect that it was fake because lanes K and L were different from ceftriaxone. One degraded sample with API content of 84% was mis-classified as good quality.

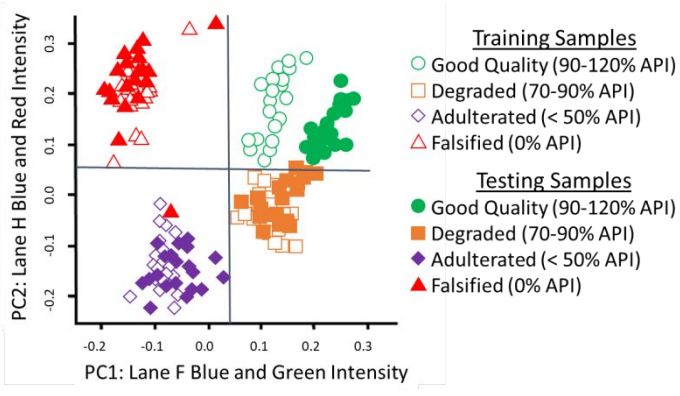

Figure 3. Principal Component Analysis (PCA) of ceftriaxone training and testing samples. Images captured with Google Pixel camera.

3.3 Blinded study with novice reader.

We tested the ability of a novice PAD reader to classify ceftriaxone samples "by eye". The tester was given images and a written description of the color barcode for good quality ceftriaxone and then asked to categorize sample images as "meets standard" or "suspicious" based off the barcode expected for good quality ceftriaxone. They marked any deviations (expected colors in lanes missing or extra lanes present) as bad quality. The reader correctly identified 78 out of 80 samples with one false positive and one false negative, resulting in 98% specificity, 95% sensitivity, and overall 97.5% accuracy.

Table 1. Evaluation of ceftriaxone quality by eye. A novice user correctly identified 98% of the samples based on comparison with stored images of good quality ceftriaxone.

Sample Type (% Ceftriaxone)	Correct Identification
Falsified (0)	29/30
Adulterated (< 50)	10/10
Degraded (70-90)	20/20
Good Quality (90-120)	19/20

The PADs used by the novice reader were also captured with the Pixel phone camera and classified using the PCA thresholds determined in section 3.2. Figure 4 and Table 1 show the accurate classification obtained. The specificity was 95% and the sensitivity was 100%, giving an overall accuracy of 98%.

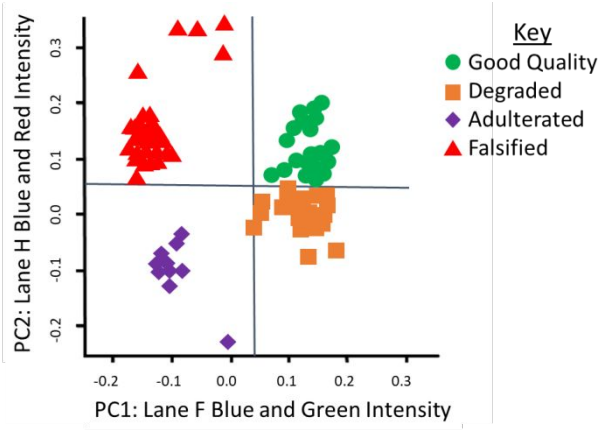

Figure 4. PCA analysis of independent set of samples. The samples from the novice PAD user matched the results shown in Figure 3. Images captured with Google Pixel camera.

3.4 Dosage Form Analysis.

Forty dosage forms collected in Western Kenya were analyzed on the PAD and by HPLC. Only four samples failed to meet USP specifications, falling at 77.8, 78.0, 80.5, and 87.1% API content. All four of the failing samples had degradation products visible in their HPLC chromatograms. The PAD correctly identified all but one of the failing samples. It missed the 87.1% sample, which was less than three percent outside of the allowed range. Figure 5 shows the PCA image analysis.

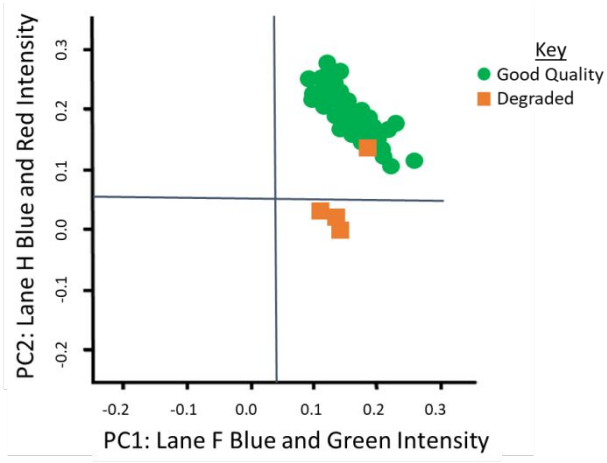

Figure 5. Evaluation of ceftriaxone quality in 40 samples collected in Kenya. The PADs had an overall accuracy of 98% for classification of the dosage forms. PADs were imaged with the Google Pixel.

3.5 Different cell phones gave different PCA outcomes

We compared two cell phones, a Google Pixel and an LG Spectrum 2, to determine whether they could be used interchangeably in classifying ceftriaxone as good or bad quality. A 110 mg/ml solution of ceftriaxone was kept at room temperature for a week, during which time the API content dropped below 50%. PAD and HPLC analysis was completed each day. To determine if the results were specific to one type of phone each PAD was imaged with a Google Pixel and an LG Spectrum 2 for subsequent image analysis. The PCA for this comparison is shown in Figure 6.

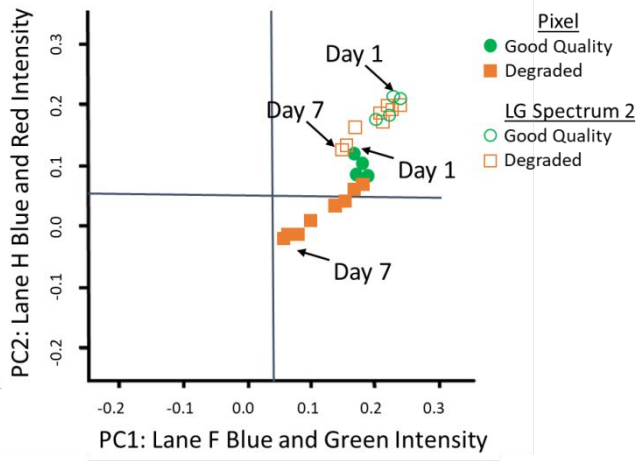

Figure 6. Phone Comparison PCA. The Google Pixel (solid dots and squares) and LG Spectrum 2 (hollow dots and squares) had an accuracy of 83% and 33%, respectively, for classifying the test solution as good quality vs. degraded.

The two samples misclassified by the Google Pixel were at 87% and 89% API content, which is just outside the allowed range. The LG Spectrum 2 images produced higher RGB values than the Google Pixel for the same PAD. Since the Google Pixel was

used for standardization it is not surprising that that LG Spectrum 2 gave a lower accuracy. The two phones were not interchangeable, and in field use, it will be necessary to provide users with a standard image collection tool or use color standards printed on the PADs to correct for different camera behaviors.

3.6 Stability of the PADs

To determine the stability of the paper test cards, groups of a dozen cards were sealed in foil-coated Ziploc bags and stored at room temperature for 0, 2, or 4 weeks. Ceftriaxone solutions containing either good quality (100% API content, n = 12) or degraded (50-90% API content, n=12) ceftriaxone were run on the PADs and then classified using the PCA metric shown in Figure 4. All of the good quality samples were correctly identified and 10/12 of the degraded samples were correctly identified; there was no change in performance over time. The degraded samples that were mislabeled at all 3 time points had API contents of 87 and 89%. These results show that the PAD is stable over at least 4 weeks at room temperature.

Discussion

The purpose of performing field tests on pharmaceuticals is to improve the quality of medicines. It is thus critical to detect substandard and falsified (SF) products as quickly as possible and identify how they have entered the supply chain. For ceftriaxone, quality problems can arise at different points in the supply chain. Failures in good manufacturing practice can lead to underdosing, adulteration, or falsification. Distribution is another critical stage. A WHO study (WHO, 1985) concluded that bad distribution and storage practices can degrade the quality of a third of the pharmaceuticals on the WHO essential drugs list. Further down the supply chain, a good-quality drug can degrade due to bad dispensing practices, such as reconstituting a vial of antibiotic and storing the solution for administration over several days. We observed auto-catalyzed base degradation of ceftriaxone in 4-5 days when reconstituted dosage forms were stored at 23˚C; in regions where room temperature is ten degrees higher, degradation will occur even faster. (Snape et al, 2010)

When the PAD is dosed with a solution of ceftriaxone and read with an image analysis program, the PAD correctly identifies substandard ceftriaxone with API content between 0-80%, and is able to discriminate accurately between products that are substandard because of degradation, and products that are substandard due to adulteration. We note that even with controlled dosing, the PAD was unable to differentiate slightly substandard ceftriaxone, with API content in the 80-90% range, from good quality ceftriaxone. The Minilab is also unable to detect pharmaceuticals with API content in this range, highlighting an area where further research is needed. (Pan and Ba-Thein, 2018)

Conclusions

The ability of the PAD to distinguish degraded, adulterated, and falsified ceftriaxone with an accuracy of 95%, combined with its low cost and ease of use, could allow routine monitoring of this essential medicine's quality all through the supply chain. In particular, this PAD test could be performed on injectable ceftriaxone prepared for clinical use. Detection of degraded, adulterated, or falsified products could save lives at the point of care, as caregivers could switch to a different brand while the suspicious product is reported to the DRA and moves through the confirmatory analysis process. For the DRA, time and money would be saved by focusing scarce confirmatory analysis capacity on products that are likely to be of bad quality.

Conflicts of interest

ML is an inventor on a US patent (US 9354181B2) issued for the paper analytical devices used in this study. The US patent is held by the University of Notre Dame and licensed to Veripad LCC. ML has no financial interests in Veripad, and there are no international patents or patent applications on the PAD technology.

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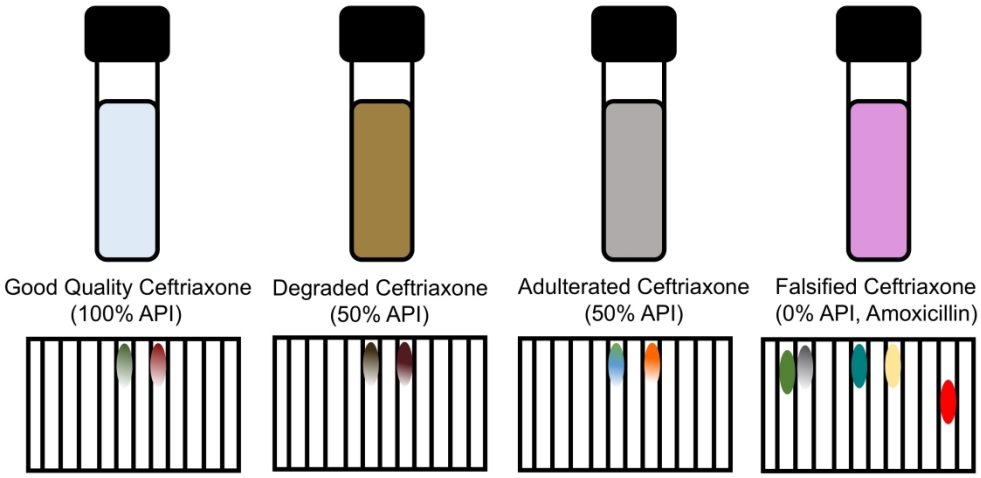
References

1 World Health Organization, WHO Model List of Essential Medicines (19th List), 2015.
2 M. De Diego, G. Godoy and S. Mennickent, *J. Chil. Chem. Soc.* 2010, **55**, 335-337.
3 World Health Organization, Reversing the Trends of the Second National Health Sector Strategic Plan. Republic of Kenya. *Clinical Management and Referral Guidelines*, 2009, Vol 3.
4 JW. Nickerson, A. Attaran, BD. Westerberg, S. Curtis, S. Overton and P. Mayer, *Morbidity and Mortality Weekly Report*, Centers for Disease Control and Prevention, 2016, **64**, 1375-1376.
5 World Health Organization, 1 in 10 medical products in developing countries is substandard or falsified. News Release. Geneva, Switzerland. 28 Nov. **2017**.
6 P. Newton, M. Green and F. Fernandez, *Trends in Pharmacol. Sci*., 2010, **31**, 99-101.
7 S. Kovacs, SE. Hawes, SN. Maley, E. Mosites, L. Wong and A. Stergachis, *PLoS ONE*, 2014, **9**(3), e90601.
8 A. Obaid, *Pak. J. Pharm. Sci*., 2009, **22**, 220-229.
9 GPHF, 2017. The GPHF-Minilab™-Protection against Counterfeit Medicines. www.gphf.org/en/minilab/index.htm
10 RWO. Jähnke, G. Küsters and K. Fleischer, *Drug Info. J.*, 2001, **35**, 941-945.
11 H. Pan and W. Ba-Then, *Am. J. Trop. Med. Hyg*. 2018, **98**(1), 344-348.

12 P. Risha, Z. Msuya, M. Ndomondo-Sigonda, T. Layloff, *J. AOAC Int.*, 2006, **89**, 1300-1304.
13 AA. Weaver, H. Reiser, T. Barstis, M. Benvenuti, D. Ghosh, M. Hunkler, B. Joy, L. Koenig, K. Raddell and M. Lieberman, *Anal. Chem.*, 2013, **85**, 6453-6460.
14 AA. Weaver and M. Lieberman, *Am. J. Trop. Med. Hyg.*, 2015, **92** (6_Suppl 3), 17-23.
15 United States Pharmacopeial Convention, Cetriaxone for Injection, Interim Revision Announcement, 2016.
16 CA. Schneider, WS. Rasband and KW. Eliceiri, *Nat Met*. 2012, **9**, 671-675.
17 RS. Bivand, E. Pebesma and V. Gomex-Rubio, *Applied Spatial Data Analysis with R*, 2013, Springer, New York, NY.
18 M. Zajac and I. Muszalska, *Acta. Pol. Pharma. Drug Res.*, 1998, **55**(1), 35-39.
19 World Health Organization, Accelerated Stability Studies of Widely Used Pharmaceutical Substances Under Simulated Tropical Conditions, 1985.
20 TJ. Snape, AM. Astles and J. Davies, Understanding the chemical basis of drug stability and degradation, *Pharma. J.*, 2010.



A paper analytical device (PAD) can classify good quality, degraded, adulterated, and falsified samples of the antibiotic ceftriaxone with 95% accuracy.

1057x530mm (72 x 72 DPI)