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In Vitro Ceftriaxone Stability at New-borns' Rectal PH Assessed by UV and HPLC Methods

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Abstract

This study showed the comparison of UV spectroscopy and High Performance Liquid Chromatography (HPLC) for ceftriaxone stability. UV spectroscopy using wavelength ratio between 241 and 271 nm absorbance values can be used successfully as a screening technique in ceftriaxone stability investigations. Ion paring reversed phase – High Performance Liquid Chromatography provided more precise stability characterization. The HPLC conditions developed were in isocratic mode using an YMC ODS H80, 150 x 4.6 mm, 4 µm with a mobile phase composed by 40% of methanol and 60% of phosphate buffer (10 mM; pH 7.5) where tetrabutylammonium bromide was solubilized at 18 mM. Detection was performed with a diode array detector from 200 to 400 nm. Sample injection volume was at 5 µL. Methanol was selected because better symmetry of ceftriaxone peak than acetonitrile was obtained. Both methods were validated.

The calibration curve and stability study was performed over a concentration range of 7.5 to 16.5 mg.L-1. 100% corresponded to the concentration of 15 mg.L-1. Intermediate precision was tested on 6 independent samples at concentrations corresponding to 100% (15 mg.L-1) on 6 consecutive days. These values were within the acceptance criteria of 2% and showed that both methods were precise. Accuracy of the method was evaluated analyzing three independent samples at concentrations corresponding to 100%. Recovery percentage calculated between the known concentration and the calculated concentration of ceftriaxone showed that the methods were accurate. Thus both methods were linear.

The stability study was performed at 40°C as infants with sepsis are generally febrile. Over the rectal pH range recorded in sick infants, the stability of ceftriaxone was maximal at pH 7.5. Over 6 hours in a pH range of 6.5 to 8.5 less than 10% of ceftriaxone is degraded. However at pH 5.5, degradation occurred more rapidly and loss of drug was significant.

Keywords: Ceftriaxone; HPLC; Stability; UV spectroscopy; Infant rectal pH; Validation; Pharmaceutical development

Introduction

Neonatal sepsis is the leading cause of preventable death in newborns living in tropical countries. Mortality can be as high as 40% [1-3].

Ceftriaxone (CFX) is a well-established third generation cephalosporin antibiotic with a broad spectrum of activity encompassing the majority of bacterial pathogens responsible for neonatal sepsis [4]. CFX is widely used in hospital practice for the treatment of severe sepsis, including central nervous system infections, at all ages. However, available commercial formulations are limited to parenteral administration (intramuscular and intravenous), which currently precludes its use in rural areas of developing countries. These areas lack health facilities where injections can be given safely. Many of the deaths from neonatal sepsis occur at or near home before the infant can reach the hospital. This same therapeutic gap confronted the community treatment of severe malaria in young children and led to the successful development of rectal artesunate as a pre-referral treatment [5]. There is an interest in developing this same paradigm for the antibiotic treatment of neonatal sepsis. Ceftriaxone has the considerable advantages of an excellent safety record, good central nervous system penetration, and much slower elimination than other third generation cephalosporin providing more than 24 hours antibiotic activity after a single administration. Concerns over the potential for biliary sludging or bilirubin displacement and kernicterus in neonates are not considered a major obstacle to the development of a single dose pre-referral treatment of a life threatening illness. This study was conducted to develop a reliable analytical method for CFX stability study at the pH values recorded in the infant rectum [6]. Mean rectal pH values in neonates were slightly lower than in older infants: 6.47 compared with 6.90, respectively with a range of 5.5 to 8.2.

The stability of CFX sodium in biological fluids has been reported in clinical studies in which samples were stored for several months before being analyzed [7,8]. The stability of CFX was determined under the necessary storage conditions. The presence of phosphate buffer at pH 6.0 and lower storage temperatures (from 25°C to -40°C) improved the stability of ceftriaxone in water and cerebrospinal fluid.

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These HPLC methods required the use of an ion-pairing agent for CFX content determination according to the European Pharmacopeia related substances monograph of CFX sodium [9]. Recently, separation of cephalosporin was attempted by HILIC [10], but this approach was not suitable for the analysis of degradation products and quantitative determination of CFX. HPLC without ion pairing provided rapid analysis [11]. Capillary zone electrophoresis is another method for the study of ceftriaxone degradation but it is very slow, and therefore unsuitable for rapid screening [12]. For degradation studies, HPLC is the most appropriate technique, however for preformulation development where concentrations are very high the use of a more rapid method such as UV spectrometry may be advantageous [13,14].

For preformulation development, there is a need for a rapid method for CFX determination content for stability assessment. Therefore in this study UV and HPLC were compared and assessed to establish CFX stability at a pH corresponding to an infant's rectal pH.

Experimental

Chemicals and reagents

Acetonitrile and methanol were isocratic HPLC grade, purchased from Prolabo VWR (Leuden, Belgium). KH_2PO_4, K_2HPO_4, sodium tetraborate, boric acid were from Merck (Darmstadt, Germany) and tetrabutylammonium bromide was from Sigma Aldrich (St Quentin Fallavier, France). HPLC grade water was obtained by taking reverse osmosis water and passing it through an Elga Milli-Q System. Ceftriaxone sodium was provided by Hoffmann-La Roche (Basel, Switzerland).

Apparatus

HPLC: The LC system consisted of a Spectra System P4000 pump, a UV 6000LP detector with path length of 50mm, an AS 3000 autosampler and a SN 4000 system controller from TSP (Courtaboeuf, France). The analytical column was an YMC ODS H80, 150 x 4.6 mm, 4 µm (Interchim, Montluçon, France). All chromatographic experiments were performed in isocratic mode. The mobile phase was pumped at a flow rate of 1mL.min^{-1} with 5 µL sample injection volume. Chromeleon software was used for chromatographic acquisition.

UV absorbance: Absorbance and spectra were recorded across the wavelength range of 200-400 nm using a Jasco V630 spectrophotometer (Jasco France, Bouguenais), under the following conditions: scan speed 100 nm.min^{-1}; data pitch 0.2 nm; spectral bandwidth 1.5 nm. Spectra Manager II software (Jasco, France) was used for spectral acquisition.

Calibration model preparation

The calibration standard set consisted of five different concentration levels (50%, 75%, 90%, 100% and 110%) of CFX solutions. 100% corresponded to 30 mg of CFX dissolved in buffer solution and a 1/20 dilution in buffer solution. Three independent replicates of calibration sets were performed on three different days to determine linearity, intermediate precision and accuracy.

Stability study

The stability study was performed in buffer solutions at 50 mM in the dark and at 40°C. pH values at 6.5 and 7.5 were obtained with phosphate buffer, and pH values at 5.5 and 8.5 were obtained with acetate and borate buffers, respectively. For these pH values, CFX is negatively charged because of ionization of the carboxylic acid group (pKa = 2.37) and the hydroxytriazinone group (pKa = 4.21) [15].

Results and Discussion

The aim of the study was to evaluate UV spectroscopy for ceftriaxone stability studies. A HPLC method was used as a comparator to validate the spectroscopic approach for stability studies.

HPLC method selection

The HPLC method was performed by ion-pairing HPLC using octadecyl grafted silica as applied mainly for CFX analysis [8,16,17]. An YMC ODS H80 column was selected to improve resolution and peak symmetry. The mobile phase was a mixture of a strong solvent and an aqueous phase composed of phosphate buffer (final concentration 10 mM) at pH 7.5 where tetrabutylammonium bromide was dissolved to reach 18 mM. At pH 7.5, the ion-pairing reagent combined with anionic CFX and formed hydrophobic ion-pair was easily retained in the reversed phase conditions. 30% acetonitrile and 40% methanol were compared as strong solvents in the mobile phase. Methanol gave better symmetry of CFX peaks.

Using UV detection, two maxima were observed at 241 and 271 nm in the pH range from 5.5 to 8.5. The detection was set at 241 nm since the sensitivity was higher at this wavelength.

UV method selection

From pH values 5.5 to 8.5, the same spectra of CFX were observed with two maxima at 241 and 271 nm assigned to λ_{max1} and λ_{max2}, respectively. Therefore for the stability study of CFX in comparison with HPLC, spectra from 200 to 400 nm were recorded and absorbance values were measured at the maxima.

Methods comparison

UV and HPLC methods were compared in term of linearity, precision and accuracy (Table 1). The calibration curve and stability study were performed over a concentration range of 7.5 to 16.5 mg.L^{-1}. 100% corresponded to the concentration of 15 mg.L^{-1}.

Intermediate precision was tested on 6 independent samples at concentrations corresponding to 100% (15 mg.L^{-1}) on 6 consecutive days. These values were within the acceptance criteria of 2% and showed that both methods were precise.

Accuracy of the method was evaluated analyzing three independent samples at concentrations corresponding to 100%. Recovery percentage calculated between the known concentration and the calculated concentration of CFX showed that the methods were accurate (Table 1).

Thus both methods were linear and therefore appropriate for determination of CFX content.

Parameter	HPLC 241 nm	UV 241 nm
Linearity	-	-
Correlation coefficient	0.9983	0.9994
Slope	65412277	0.0485
Y intercept	– 5201	– 0.0014
Intermediate precision RSD %, n = 6	1.5	0.8
Accuracy (100%) Average recovery % RSD %, n = 6	100.2 0.9	100.4 0.8

Table 1: Validation Parameters.

Stability study

As infants with sepsis are generally febrile, the stability study was performed at 40°C. This temperature was selected as the worst case. It is known that CFX degradation is reduced by decreasing temperature [8,17]. Organic impurities can originate from degradation products which may depend on alterations in reaction conditions such as temperature or pH. At 40°C, CFX stability was established by HPLC and UV spectroscopy between pH values range from 5.5 to 8.5 which correspond to the range of infants rectal pH values.

The advantages of HPLC were the CFX determination content and the observation of degradation products which provided relevant results for the stability observation. The results of CFX content established by HPLC show that the greatest stability was reached at pH 7.5 (Figure 1) and the worst was at pH 5.5. The average rectal pH values are6.5 and 6.9 for neonates and older infants, respectively. Figure 1b shows that more than 90% of CFX remains stable at pH values between 6.5 to 8.5 after 6 hours. As it is unlikely that the residence time of a CFX rectal formulation would be longer than 6 hours, due to neonates frequent defecation, intrarectal CFX degradation is unlikely to be a major factor affecting stability.

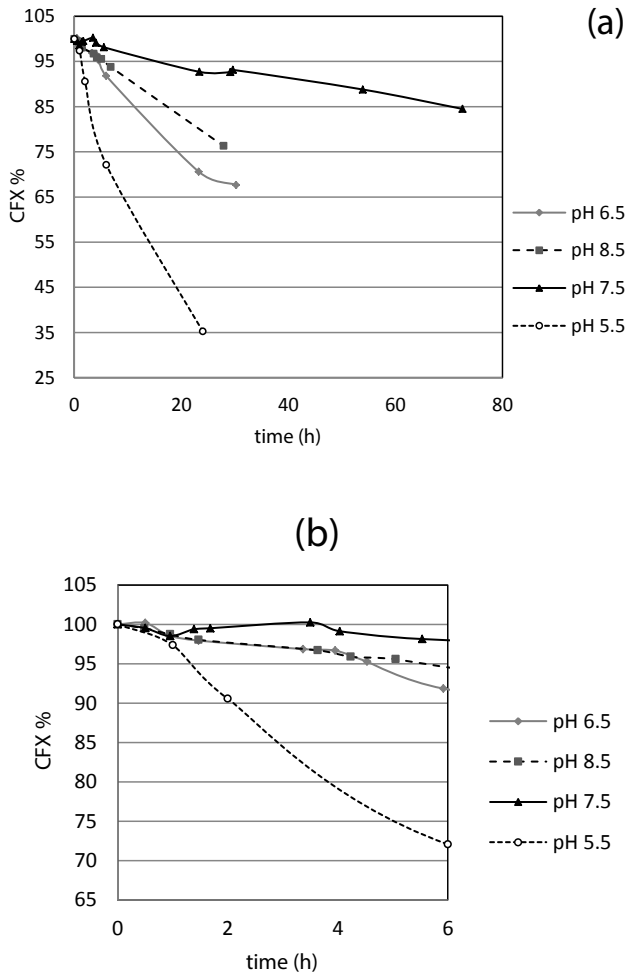

Figure 1: CFX stability at different pH conditions at 40°C assessed by HPLC; (b) the zoom of (a).

UV spectroscopy was assessed for studying CFX stability of the same samples. The advantage of UV spectroscopy was the rapidity of analysis. Different approaches were envisaged, one by measurement of absorbance values at the maxima of CFX and another one by calculating the ratio R between $A_{\lambda max1}$ and $A_{\lambda max2.}$ HPLC established that CFX degradation was observed at 6h, therefore results obtained with UV spectroscopy are presented only until 6 h in Figure 2. Measurement at constant wavelength values (Figure 2a and 2b) showed important variations with increases and decreases at 241 and 271 nm, respectively, at 5.5 pH value. Therefore change in absorbance values was insufficient as to underscore the degradation. Absorbance

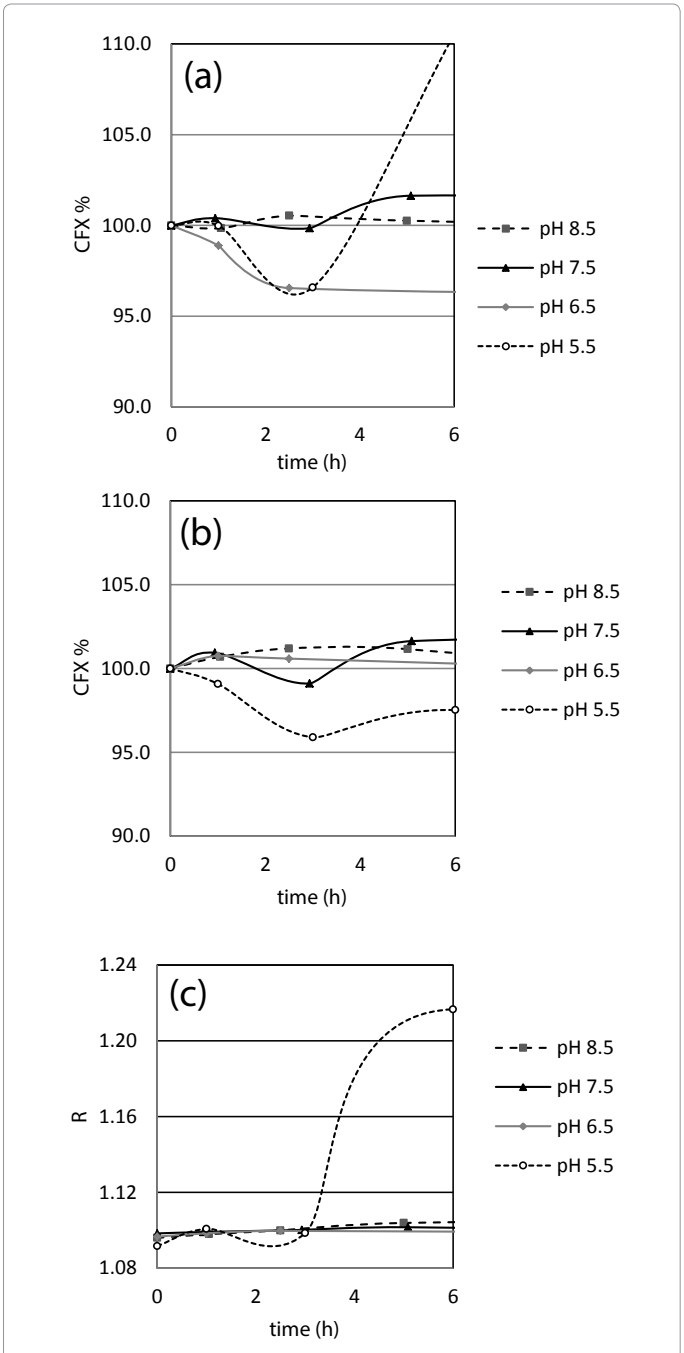

Figure 2: CFX stability at different pH conditions at 40°C assessed by UV; (a) 241nm; (b) 271nm; (c) $R=A_{241}/A_{271}$.

value ratios ($R = A_{241}/A_{271}$) were more informative. Figure 2c clearly shows CFX degradation at 3h at a pH of 5.5.

Degradation products are pH dependent as shown by the HPLC analysis in Figure 3. At acidic and neutral pH (6.5 and 7.5, respectively), two degradation products were observed at retention times lower than that observed for CFX (peaks 1 and 2 around t_r = 3.5 min). Whereas at basic pH (8.0), the degradation product represented by peak 1 was not present and a third degradation product was observed at a retention time higher than that of CFX (peak 3 around t_r = 10 min). Figure 4 shows the UV spectra of each degradation product. The degradation product corresponding to peak 2 had a spectrum with two bands at values close to those of CFX. This product was present in the raw material at content under 0.1%.

Conclusion

This study shows that UV spectroscopy can be used successfully as a screening technique in CFX stability investigations. HPLC provides more precise stability characterization.

Over the rectal pH range recorded in sick infants, the stability of CFX was maximal at pH 7.5. Over 6 hours in a pH range of 6.5 to 8.5 less than 10% of CFX is degraded. However at pH 5.5, degradation occurred more rapidly and loss of drug was significant. In a previous study, the greatest stability of CFX was observed over a pH range between 4 to 6 [18]. However that study was performed using RP-HPLC without an ion pairing agent, so CFX was poorly retained. A different study [17] showed good stability of CFX at pH 7.4 using an ion-pair RP-HPLC assay.

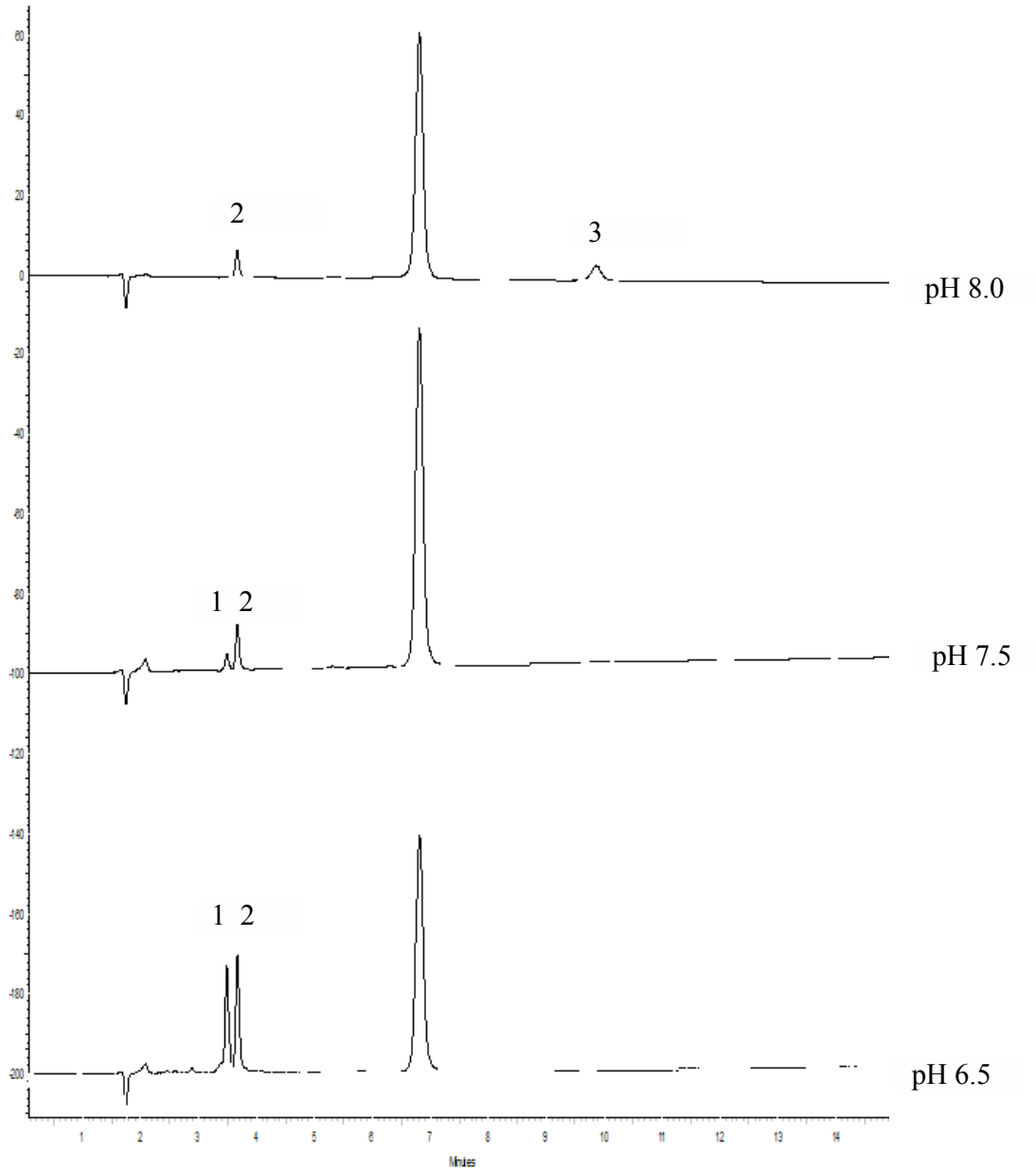

Figure 3: Retention of degradation products obtained at pH 6.5, 7.5 and 8.0 after 24 h.

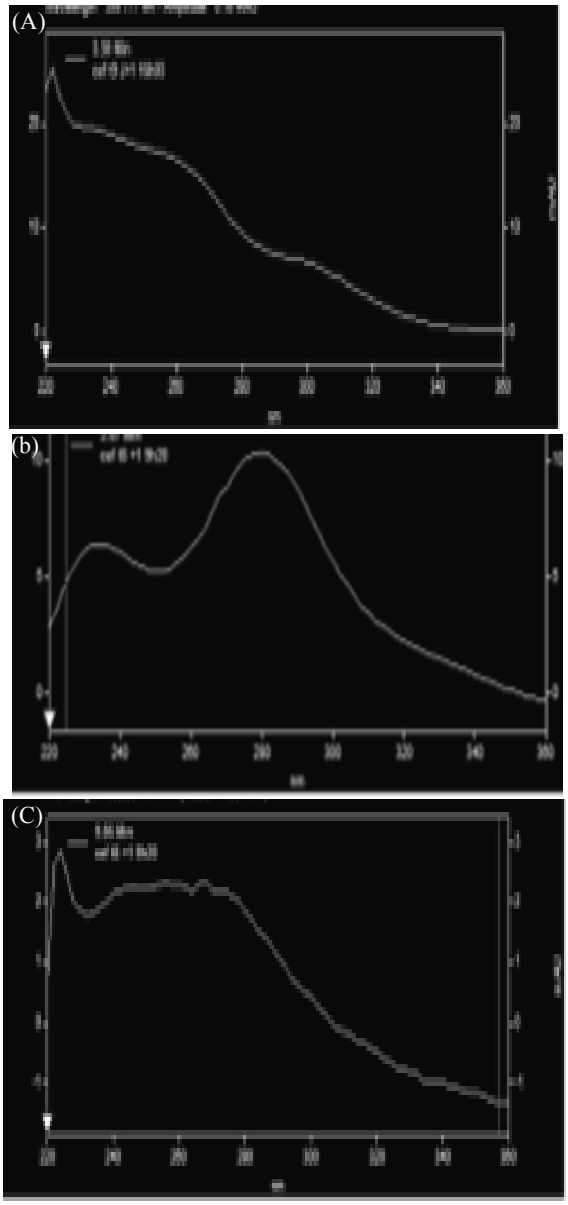

Figure 4: UV spectra of degradation products obtained: (a), (b) and (c) spectrum corresponding to peak 1, 2 and 3 on chromatogram.

Intrarectal pH mediated degradation of CFX is unlikely to be of therapeutic importance. Further studies of CFX stability are needed for formulation development. An excipient which controls pH may be of interest for the development of a rectal formulation of CFX. The rapid UV method could be used to screen interactions between CFX and excipients, but HPLC will be required for characterization and separation of CFX and degradation products.

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M. Gomes is a staff member of the WHO; the authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the WHO.

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