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Contribution of unsuitable packaging and uncontrolled circuits and exposures to the poor quality of medicines collected in sub-Saharan countries

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5 1 Contribution of unsuitable packaging and uncontrolled circuits
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22 Abstract

23 Introduction

24 The incidence of cardiovascular diseases is increasing and there is a growing need to provide
25 access to quality cardio drugs in Africa. In the SEVEN study, we analysed 1530 cardiovascular
26 drug samples randomly collected from ten African countries. By that time, of the seven drugs
27 products analysed, only those containing amlodipine and captopril had very low assay values
28 with active substance contents that could be less than 75% of those expected. In this article
29 we investigate complementary aspects of the amlodipine and captopril samples so to explain
30 the previously observed low assays for these two drugs.

31 Methods

32 The chromatograms of drug products (amlodipine : n=305 ; captopril : n=235) obtained during
33 the SEVEN study were reprocessed to quantify the relative amounts (% w/w) of impurities and
34 assign their structures. Identification of the main compounds of the drugs packaging was
35 performed by use of infrared spectroscopy.

36 Results

37 Chromatogram analysis showed that 40 amlodipine tablets and 31 captopril tablets contained
38 at least one impurity exceeding the recommended thresholds. The low levels previously
39 observed in several samples could be the result of the use of a degraded drug substance in
40 the manufacture and/or degradation of the drug product due to uncontrolled exposure.

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3 41 Identification of the blister packaging of the samples led to separate both amlodipine and
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5 42 captopril drug samples in two groups. Mann Whitney's bilateral test showed a significant
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8 43 difference ($p < 0.0001$) between the median value of the captopril dosage when tablets are
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10 44 packaged in blisters providing higher protection to humidity ($n=105$) as opposed to the tablets
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13 45 packaged in blisters providing lower humidity protection ($n=130$).

16 46 Conclusion

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20 47 Based on these results, particular attention should be paid to the materials and types of
21
22 48 packaging used in order to minimize the lack of control over the exposures and drug circuits
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25 49 present in these different countries.

29 50 Article summary : strengths and limitations of this study

- 33 51 • Complementary investigation of results obtained during the Seven study, during which
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35 52 1530 cardiovascular drug samples were prospectively collected in licensed and
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38 53 unlicensed places of sale in Africa
- 40 54 • The specifically low amounts for two drugs, amlodipine and captopril, may be due to
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43 55 degradation during storage and lack of drug protection of these sensitive drugs
- 44
45 56 • Degradation products and impurities quantities were above the recommended
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48 57 thresholds in some amlodipine and captopril samples, raising potential concern about
49
50 58 the toxicity of the drugs
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53 59 • Packaging providing high protection from residual humidity could be a leverage to
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55 60 reduce the presence of degraded amlodipine and captopril drugs on the African soil

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3 61 • Other studies should confirm, on these two sensitive drugs and other, that packaging
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5 62 providing better protection could be a mean to provide safer drugs in Africa
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10 63 1. Introduction

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14 64 Substandard drugs generally pose a serious health concern from several perspectives and this
15
16 65 is particularly prevalent in developing countries where control regulations are poorly
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18 66 developed. Numerous cases of quality defects have been reported^{1,2}, mostly in connection
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20 67 with anti-infective drugs³. However, since cardiovascular diseases are currently the most
21
22 68 important non-communicable diseases in most low- and middle-income countries, it is critical
23
24 69 to ensure that these diseases are addressed in a comprehensive manner⁴. Thus, under the
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26 70 impulse of X. Jouven, the study named SEVEN has been set up with the aim of evaluating the
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28 71 quality of the cardiovascular drugs present in ten African countries⁵⁻⁷, by exploring the case
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30 72 of 7 commonly used cardiac drugs, namely acenocoumarol, simvastatin, furosemide,
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32 73 hydrochlorothiazide, captopril, atenolol and amlodipine. Examination of the identity and assay
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34 74 of the active substances in the various samples collected revealed that among the products
35
36 75 tested, only those based on amlodipine and captopril had very low active substance content
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38 76 that could be less than 75% of those expected⁶. The observation that the biggest quality
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40 77 defects were highlighted and circumscribed around these two molecules motivated the team
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42 78 to seek additional information in particular regarding their stability as well as related elements
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44 79 such as packaging, version of drug (i.e. generic versus brand) and place of manufacture.
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54 80 For drugs where the quality defect is unintentional, according to Johnston et al, low dosage
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56 81 levels of active substance may be the result of a variety of factors, including inadequate
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58 82 package design or quality². Yet, despite the fact that the influence of packaging changes on
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3 83 the stability of medicinal products is now well established^{8,9}, to the best of our knowledge,
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5 84 the studies of the quality of real field samples have so far focused only on dosage units without
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8 85 taking into account the type of packaging used or their chemical composition³. However, the
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10 86 concept of packaging is equally important to assess, especially since the drug products need
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13 87 to be kept stable in difficult climatic conditions, where, for example, residual moisture can
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15 88 reach 88% in sub-Saharan Africa¹⁰ and the ability of a plastic blister pack to protect a drug
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17 89 from moisture is highly dependent on its design and composition¹¹.

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21 90 It is in this context that this study was taking place considering samples of captopril and
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23 91 amlodipine products, collected as part of the SEVEN study⁵⁻⁷, as tracer products. On these
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25 92 various samples was carried out the search for degradation products and/or chromatographic
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27 93 related substances as well as the identification of the packaging actually present on the
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29 94 products collected in several African countries, by trying, where possible and relevant, to
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31 95 reconcile the different data and make them meaningful in relation to the potential causes of
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33 96 underdosing previously highlighted for these two drugs.
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40 97 2. Material and method

45 98 2.1 Sample collection

46 99 The methodology and design of the samples collection are described in detail in the articles
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48 100 published in the context of the SEVEN study⁵⁻⁷. A multidisciplinary collaborative team of
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50 101 epidemiologists, cardiologists and pharmacists from France and Africa conceived and
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52 102 designed the study. It was registered with the French national drug agency (Agence Nationale
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54 103 Sécurité du Médicament ID_RCB:2014-A01275-42).

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3 104 1530 drugs samples, including 235 captopril and 305 amlodipine drug samples, were collected
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6 105 as per the Guidelines for Field Surveys of the Quality of Medicines¹². 10 countries were
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8 106 concerned: Benin, Burkina Faso, Congo, Democratic Republic of the Congo, Guinea, Côte
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10 107 d'Ivoire, Mauritania, Niger, Senegal and Togo. In these countries, samples were obtained from
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12 108 licensed (pharmacy) sources selected randomly and unlicensed (street market) places of sale
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15 109 chosen as per the local investigator's convenience. Drug samples were purchased in the
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17 110 capital city and when possible in one city located close to the country's border. Medicines
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19 111 were obtained by the study investigator's staff who posed as customers. For each drug
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21 112 sample, investigators were asked to collect generic versions and brand name versions of the
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23 113 drug if available. After purchase, all drugs were stored at ambient temperature, in a dry place
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25 114 avoiding direct sunlight. Samples were sent via courier to the coordinating centre in France.
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31 115 2.2 Reagents and sample preparation

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33 116 Amlodipine besylate, captopril, iodine, sodium thiosulfate and triethylamine (>99% pure)
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35 117 were purchased from Sigma Aldrich (St. Quentin Fallavier, France). Analytical grade
36
37 118 acetonitrile and methanol came from VWR Prolabo® Chemicals (Fontenay-sous-Bois, France).
38
39 119 Ultrapure water was produced by the Q-Pod Milli-Q® system (Millipore, Molsheim, France).
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41 120 The stationary phase consisted of a Kinetex® (Phenomenex, Torrance, U.S.A.) C18 column (4.6
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43 121 mmx250 mm, i.d., 5 µm).
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49 122 Standard solutions were prepared as per the recommendations provided in the United States
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51 123 Pharmacopeia (USP) monograph of amlodipine besylate and captopril tablets. Captopril
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53 124 disulfide was obtained by using the protocol proposed in the European Pharmacopeia (Ph.
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55 125 Eur.) monograph of captopril.
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126 2.3. Analytical conditions and data analysis

127 Chromatographic analyses were performed using a Dionex Ultimate 3000 system (DIONEX,
128 Ulis, France) coupled to a diode array detector. Chromatographic conditions as well as
129 suitability tests were performed as per the recommendations provided in the monograph of
130 amlodipine tablet (USP) and captopril (Ph. Eur.).

131 Identification of blisters was achieved using Fourier Transformed Infrared Spectroscopy (FTIR).
132 FTIR Perkin-Elmer Spectrum 2000® spectrometer (Villebon-sur-Yvette, France) with a diamond
133 crystal. Resolution, scan range and number of accumulated scans per spectrum were set to
134 0.5 cm^{-1} , 4000 to 400 cm^{-1} and 3, respectively. Spectrum were acquired on each side of the
135 blisters using attenuated total reflectance mode.

136 All statistical tests were performed using GraphPad Prism version 8.3.1. for Windows
137 (GraphPad Software, La Jolla California, USA). Nonparametric Spearman's correlation
138 coefficient (r) was calculated to measure the strength of the association between the peak
139 surface area of captopril and that of captopril disulfide. Mann-Whitney tests were used to
140 compare the median between drugs with lower and those with higher protection from
141 humidity packaging blisters.

142 3. Results and discussion

143 3.1. Chromatographic purity profiles of the drugs

144 3.1.1. Captopril samples

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3 145 The qualitative study of the chromatograms of the 235 captopril drug samples showed that
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6 146 the 31 samples with drug amounts inferior to 90% (w/w%) contained the same impurity (Fig.
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8 147 1). The detected impurity was assigned to captopril disulfide as it had the same retention time
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10 148 than this compound.

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14 149 Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance
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16 150 standard solution (black), typical substandard drug sample (blue).

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20 151 Further, for every chromatogram, the sum of the surface of the peak areas corresponding to
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22 152 captopril and that of captopril disulfide led to obtain areas recoveries between 90 and 110%
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24 153 compared to that of the captopril reference solution. Therefore, one could infer that the lack
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26 154 of drug captopril may be linked to the presence of captopril disulfide. As a result, a correlation
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28 155 study between the surface of the peak of captopril and that of captopril disulfide impurity was
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30 156 carried out and yielded a negative Spearman coefficient with a value of -0.613 ($p=0.0069$).

36 157 3.1.2. Amlodipine samples

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39 158 Unlike captopril, the 305 chromatographic profiles obtained for amlodipine samples strongly
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41 159 differed from one to the other, implying that the drugs did not contain the same impurities
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43 160 (Fig. 2). Indeed, under the analytical conditions recommended in the USP monograph, two
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45 161 categories of chromatographic profiles were obtained as a function of the studied samples.

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50 162 A first category of chromatographic profiles consisted of samples containing an impurity with
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52 163 a relative retention time of about 0.5 (Fig 2. a) as compared to amlodipine, referred in the USP
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54 164 as amlodipine related compound A. In 10 chromatograms, the surface of the peak of this
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56 165 impurity exceeded the USP acceptance criteria for this impurity (<1.0 %, w/w).

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3 166 The second category of chromatographic profiles comprised a peak with a relative retention
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5 167 time of about 0.8 (Fig 2.b) corresponding to a degradation related impurity, namely an
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8 168 amlodipine-lactose interaction product. For 30 sample solutions, the area of the peak of this
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10 169 impurity compared to that of amlodipine exceeded the USP acceptance criteria for this
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13 170 impurity (<0.5% w/w).

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16 171 Fig. 2 : typical chromatographic profiles for samples containing impurity A (a) and samples
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19 172 containing amlodipine-lactose interaction product (b)

20 21 22 23 173 3.2. Blisters identification

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26 174 The identity and aspect of the blisters materials strongly differed between amlodipine and
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29 175 captopril and within drug samples of the same drug.

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32 176 FTIR analysis were performed on each side of the plastic blisters in order to identify its main
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35 177 compounds. Based on the analysis, two groups could be established: those with a spectrum
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38 178 corresponding to polyvinyl-chloride's one (PVC, red and blue spectrum, Fig. 3) and those which
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41 179 have two spectral bands (597 and 527 cm^{-1}) characteristic of the polyvinylidene-chloride
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43 180 presence¹³ (PVDC, green spectrum, Fig. 3).

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46 181 Fig. 3 : typical FTIR spectrum of a PVC polymer packaging with (green spectrum) and without
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49 182 (blue and red spectrum) PVDC.

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52 183 The analysis highlighted that 130 out of the 235 captopril drug samples were packaged in
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55 184 blisters containing PVDC and 105 in blisters only composed of PVC. In the case of amlodipine,
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58 185 none of the plastic blisters used to package 245 amlodipine samples contained PVDC (Table
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3 186 1). 60 amlodipine samples were packaged in blisters consisting of foil-foil sealed aluminium
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5 187 (Table 1).
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9 188 Table 1 : number of amlodipine and captopril drug samples as a function of blister identity
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Blister identity	Amlodipine	Captopril
PVC only	60	105
Aluminium only	245	None
PVC-PVDC	None	130

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26 190 3.3. Drugs assays as a function of blister identifications

27 191 From the results obtained in 3.2., captopril assays results were separated based on the
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29 192 presence of PVDC or not (PVC only). Amlodipine assay results were divided in two groups: the
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31 193 group of samples packaged in foil-foil sealed aluminium blisters and the one packaged in PVC
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33 194 blisters (PVC only). The results are provided in figure 4.
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41 195 Fig.4 : medians of drug assays with interquartile ranges as a function of the drug and the blister
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43 196 composition, ns: non statistically significant; ****: $p < 0.0001$.
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46 197 For amlodipine, no statistical difference in dosage ($p = 0.7684$) was observed between the
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48 198 group with foil-foil aluminium packaging (median=96.10, $n = 60$) and the group with PVC blister
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50 199 packaging (median=96.60, $n = 245$).
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54 200 For captopril, on the other hand, a statistical difference ($p < 0.0001$) was found between the
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56 201 group of samples stored in blisters containing only PVC (median=100.0, $n = 105$) and those
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58 202 stored in blisters containing PVC/PVDC (median=96.60, $n = 130$).
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4. Discussion

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204 Sub-standard cardiac medicines represent a serious health hazard and result in significant
205 morbidity and mortality⁴. The SEVEN study was the first major study of cardiovascular drugs
206 in Sub-Saharan Africa⁵⁻⁷. The analysis of samples collected in Africa and through various sales
207 spots of all kinds showed that among the products used in cardiovascular diseases, about 15-
208 20% were of low quality, and the prevalence of poor quality is higher for the products
209 containing amlodipine and captopril. Given this observation and the fact that these two active
210 substances are particularly sensitive to humidity and heat, the question arose as to whether
211 the intrinsic stability of the latter had contributed more to the quality defects highlighted at
212 the time. So, in this follow-up study, we have sought to provide some answers to this question
213 by focusing on the search of associated degradation products and the nature of the packaging
214 used at the time of the on-site collections.

215 We therefore sought to supplement the assay results with chromatographic purity studies on
216 samples of the various cardiological drugs samples collected during the SEVEN study. We
217 noted that the levels of impurities are particularly high for captopril and amlodipine samples
218 (>0.5% compared to the considered active substance), as is their high prevalence of
219 underdosing relative to other products, as previously mentioned. Of course, this problem
220 could have been caused by the use of poor-quality active ingredient. But the fact that it is
221 specific to these two active substances did not allow us to limit to this aspect alone. We
222 therefore also turned our attention to the stability issue and to what goes hand in hand with
223 this aspect. The identification of chromatographic impurities present concomitantly with the

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3 224 active substance was carried out to support our hypotheses related to the degradation of
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5 225 these compounds.
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9 226 In the case of the captopril drug samples, the compound detected (Fig. 1) in addition to
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11 227 captopril was identified as captopril disulfide, a well-known degradation product of captopril
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13 228 that is prompt to be formed under moisture and heat¹⁴. In other words, this would mean that
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15 229 the underdoses initially observed in the captopril products⁶ could be attributed to the poor
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17 230 quality of the active substance used and/or, to a larger extent, to degradation occurring either
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19 231 during manufacture and/or during improper storage. However, as it is well depicted that
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21 232 captopril is far more susceptible to degradation in the presence of some excipients than alone
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23 233 ^{15,16}, one can fathom that the presence of captopril disulfide may more likely result from
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25 234 degradation due to uncontrolled exposure. As for amlodipine, chromatographic analyses
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27 235 showed in 30 samples the presence of an impurity due to amlodipine-lactose interaction (Fig.
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29 236 2 b). Such interaction was shown to occur in time and mostly under high humidity conditions¹⁷,
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31 237 which could, to some extent, explain the poor assays observed in SEVEN study⁷.
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39 238 These results are consistent with data in the literature in that the moisture vapour
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41 239 transmission rate¹⁸ (MVTR) must be controlled as it is critical to the stability of captopril¹⁴ and
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43 240 amlodipine¹⁹. The corollary is that we have indeed shown, in the case of captopril, a significant
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45 241 difference ($p < 0.0001$, Fig. 4) in active substance content between tablets extracted from PVC
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47 242 blisters and those from PVC-PVDC blisters that exhibit lower MVTR¹⁸. The results for
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49 243 amlodipine are not statistically conclusive, but somehow the trend is noticeable since samples
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51 244 with a peak area of the amlodipine-lactose interaction product greater than 0.5% (w/w) were
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53 245 exclusively from the blisters with higher MVTR i.e. the blisters composed of PVC as opposed
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55 246 to the foil-foil aluminium ones¹⁸. Accordingly, it can be seen that the use of low moisture
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3 247 barrier packaging for these two products among the different cardiac drugs tested appears to
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5 248 have contributed to their low assay values.
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9 249 This poor quality, represented by both a lower active substance content and the presence of
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11 250 degradation products, subjects the patient to safety risks. On the one hand, any dose lower
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14 251 than the dose to which the body has developed tolerance may induce an effect that is difficult
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16 252 to predict and may even be contrary to the action of the usual dose²⁰ and on the other hand,
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19 253 the degradation products formed may be toxic²¹.
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22 254 Captopril disulphide is a well-known metabolite of captopril, which can reversibly interconvert
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25 255 into captopril in the blood²². However, in human pharmacokinetic studies the amount of
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27 256 captopril disulphide in the blood never exceeds 8% of that of captopril^{22,23}. Therefore, clinical
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30 257 consequences of exposure to higher amounts of captopril disulfide are unknown. This is to be
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32 258 considered because the disulfide metabolites of sulfur-based drugs were 50 to 100 times more
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35 259 toxic on hepatocytes from isolated rats than the parent drugs²⁴.
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38 260 In some amlodipine samples, two main degradation products were detected as mentioned
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41 261 earlier, amlodipine impurity A that corresponds to the oxidation of its dihydropyridine ring
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43 262 and the amlodipine-lactose interaction product. Impurity A has been reported to be among
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45 263 the most found metabolites in urines (7%)²⁵. As its level was always inferior to 2% relative to
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48 264 the active substance in the samples analysed, one can assume that this presence may have a
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51 265 limited impact to the patients. As for the amlodipine-lactose interaction product, no data are
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53 266 currently available on its fate in the body and its toxicity, which makes it a risk to consider.
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56 267 Overall, on the basis of these additional results from the study of the chromatographic profiles
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59 268 of the collected samples, significant quantities of cardio drugs sold on African soil may not
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3 269 only present a lack of efficacy but also safety issues, due to the presence of impurities and/or
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5 270 degradation products resulting from poor storage and packaging conditions.
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10 271 Conclusion

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12 272 Further studies of captopril and amlodipine samples from Africa showed that their low assays
13
14 273 would result in part from the degradation of the active substance, which may indicate that
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17 274 protective measures to avoid degradation of the drug products were not commensurate with
18
19 275 their unstable behaviour, particularly in the presence of moisture and heat. FTIR analyses of
20
21
22 276 the samples' blisters disclosed they differ in material composition for a same active substance.
23
24 277 In the case of captopril, statistical analysis has shown that the active substance content of
25
26
27 278 products in tighter blister packs is higher than that of products in less moisture-resistant
28
29 279 packaging. In the case of amlodipine, only the samples with low moisture resistant packaging
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31
32 280 had high amounts (>0.5% w/w) of degradation products.
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34

35 281 Using packaging with elevated moisture barrier properties could be a significant leverage to
36
37 282 reduce the prevalence of substandard drugs in countries where medicines circuit and storage
38
39 283 are poorly controlled. It is therefore also important to make the health authorities in these
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41
42 284 countries aware that the quality of packaging must also be taken into account when evaluating
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45 285 medicines supplied.
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49 286 Footnotes

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51
52 288 All authors have substantial contributions.

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9 295 Drafting of the manuscript: all authors.
10
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12

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22 302 Competing interests

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28 304 Patient consent for publication

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33 306 Data sharing statement

- 34 307 No additional data available.
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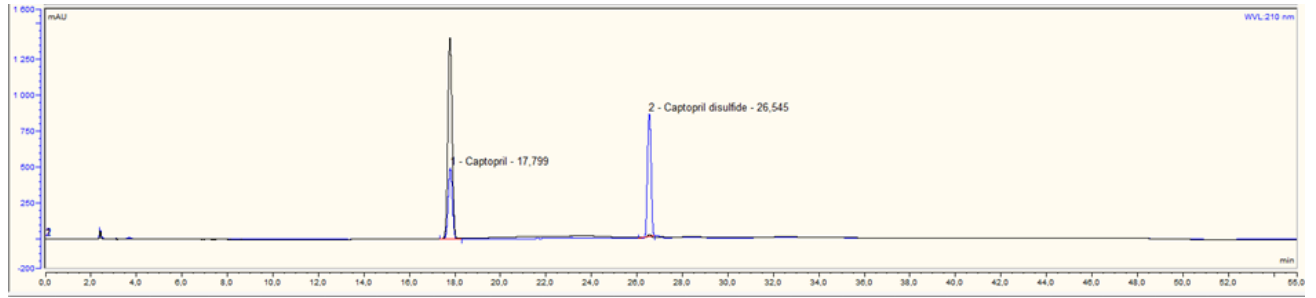


Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance standard solution (black), typical substandard drug sample (blue).

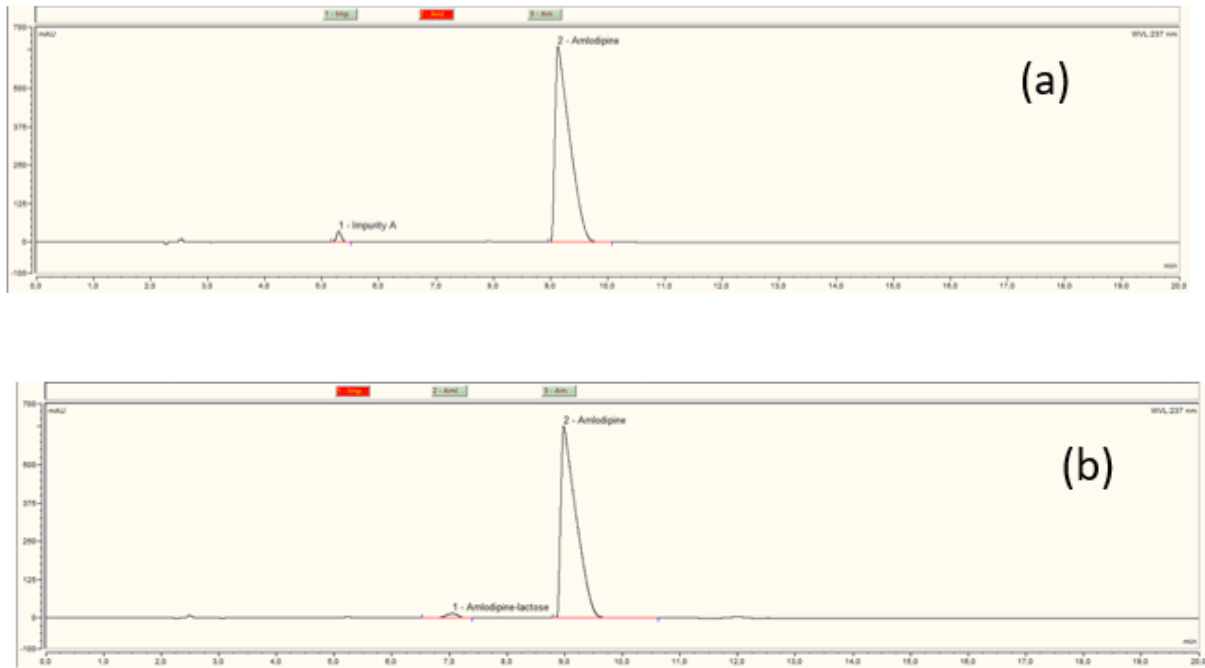


Fig. 2 : typical chromatographic profiles for samples containing impurity A (a) and samples containing amlodipine-lactose interaction product (b)

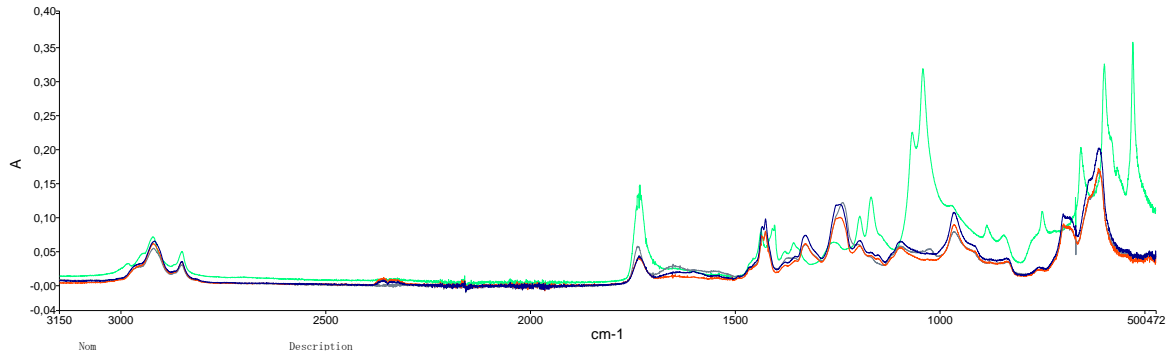


Fig. 3 : typical FTIR spectrum of a PVC polymer packaging with (green spectrum) and without (blue and red spectrum) PVDC.

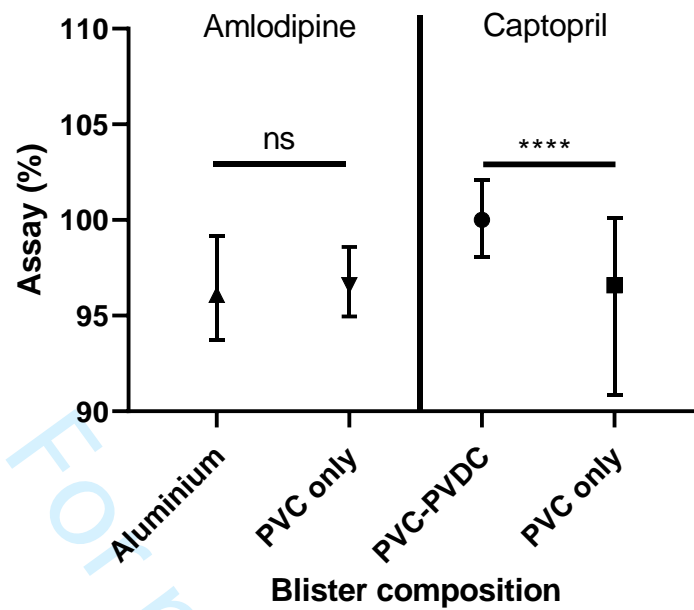


Fig.4 : medians of drug assays with interquartile ranges as a function of the drug and the blister composition, ns: non statistically significant; ****: $p < 0.0001$.

BMJ Open

A post hoc study to investigate the potential causes of poor quality of cardiovascular medicines collected in sub-Saharan countries

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23 Abstract

24 Objectives

25 The incidence of cardiovascular diseases is increasing and there is a growing need to provide
26 access to quality cardio drugs in Africa. In the SEVEN study, we analysed 1530 cardiovascular
27 drug samples randomly collected from ten African countries. By that time, of the seven drugs
28 products analysed, only those containing amlodipine and captopril had very low assay values
29 with active substance contents that could be less than 75% of those expected. In this article
30 we investigate complementary aspects of the amlodipine and captopril samples so to explain
31 the previously observed low assays for these two drugs.

32 Design

33 Post-hoc analysis of the captopril and amlodipine drugs samples and their packages collected
34 in the context of the SEVEN study.

35 Setting

36 10 countries were concerned: Benin, Burkina Faso, Congo, Democratic Republic of the Congo,
37 Guinea, Côte d'Ivoire, Mauritania, Niger, Senegal and Togo.

38 Studied drug samples

39 305 amlodipine and 235 captopril drug samples collected during the SEVEN study along with
40 their packaging were studied.

41 Outcome measures

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3 42 The drug amount and the relative amounts of drug impurities as well as the main compounds
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5 43 of the drugs packaging were analysed.
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9 44 Results

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12 45 Identification of the blister packaging of the samples led to separate both amlodipine and
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14 46 captopril drug samples in two groups. Mann Whitney's bilateral test showed a significant
15
16 47 difference ($p < 0.0001$) between the median value of the captopril dosage when tablets are
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18 48 packaged in blisters providing higher protection to humidity ($n=105$) as opposed to the tablets
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20 49 packaged in blisters providing lower humidity protection ($n=130$).
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25 26 50 Conclusion

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29 51 Based on these results, particular attention should be paid to the materials and types of
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31 52 packaging used in order to minimize the lack of control over the exposures and drug circuits
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33 53 present in these different countries.
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39 54 Article summary : strengths and limitations of this study

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43 55 • Complementary investigation of results obtained during the Seven study, during which
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45 56 1530 cardiovascular drug samples were prospectively collected in licensed and
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47 57 unlicensed places of sale in Africa
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49 58 • The specifically low amounts for two drugs, amlodipine and captopril, may be due to
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51 59 degradation during storage and lack of drug protection of these sensitive drugs
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53 60 • Degradation products and impurities quantities were above the recommended
54
55 61 thresholds in some amlodipine and captopril samples, raising potential concern about
56
57 62 the toxicity of the drugs
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- 63 • Packaging providing high protection from residual humidity could be a leverage to
64 reduce the presence of degraded amlodipine and captopril drugs on the African soil
- 65 • Other studies should confirm, on these two sensitive drugs and other, that packaging
66 providing better protection could be a mean to provide safer drugs in Africa

67 1. Introduction

68 Substandard drugs generally pose a serious health concern from several perspectives and this
69 is particularly prevalent in developing countries where control regulations are poorly
70 developed. Numerous cases of quality defects have been reported^{1,2}, mostly in connection
71 with anti-infective drugs³. However, since cardiovascular diseases are currently the most
72 important non-communicable diseases in most low- and middle-income countries, it is critical
73 to ensure that these diseases are addressed in a comprehensive manner⁴. Thus, under the
74 initiative of X. Jouven, the study named SEVEN has been set up with the aim of evaluating the
75 quality of the cardiovascular drugs present in ten African countries⁵⁻⁷, by exploring the case
76 of 7 commonly used cardiac drugs, namely acenocoumarol, simvastatin, furosemide,
77 hydrochlorothiazide, captopril, atenolol and amlodipine. It was showed that branded drugs
78 were less likely to be of poor quality compared to generic or medicines with unknown version
79 ($p < 0.001$) ; conversely place of sale was not significantly associated with the proportion of
80 poor quality ($p = 0.29$)⁶. Examination of the identity and assay of the active substances in the
81 various samples collected revealed that among the products tested, only those based on
82 amlodipine and captopril had very low active substance content that could be less than 75%
83 of those expected⁶. The observation that the biggest quality defects were highlighted and

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3 84 circumscribed around these two molecules motivated the team to search for additional
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5 85 information in particular regarding their stability.
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9 86 For drugs where the quality defect is unintentional, according to Johnston et al, low dosage
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11 87 levels of active substance may be the result of a variety of factors, including inadequate
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13 88 package design or quality². Yet, despite the fact that the influence of packaging changes on
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15 89 the stability of medicinal products is now well established^{8,9}, to the best of our knowledge,
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17 90 the studies of the quality of real field samples have so far focused only on dosage units without
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19 91 taking into account the type of packaging used or their chemical composition³. However, the
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21 92 concept of packaging is equally important to assess, especially since the drug products need
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23 93 to be kept stable in difficult climatic conditions, where, for example, residual moisture can
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25 94 reach 88% in sub-Saharan Africa¹⁰ and the ability of a plastic blister pack to protect a drug
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27 95 from moisture is highly dependent on its design and composition¹¹.
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34 96 It is in this context that this study was taking place considering samples of captopril and
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36 97 amlodipine products, collected as part of the SEVEN study⁵⁻⁷, as tracer products. On these
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38 98 various samples was carried out the search for degradation products and/or chromatographic
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40 99 related substances as well as the identification of the packaging actually present on the
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42 100 products collected in several African countries, by trying, where possible and relevant, to
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44 101 reconcile the different data and make them meaningful in relation to the potential causes of
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46 102 underdosing previously highlighted for these two drugs.
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53 103 2. Material and method

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104 2.1. Sample collection

105 The methodology and design of the samples collection are described in detail in the articles
106 published in the context of the SEVEN study⁵⁻⁷. A multidisciplinary collaborative team of
107 epidemiologists, cardiologists and pharmacists from France and Africa conceived and
108 designed the study. It was registered with the French national drug agency (Agence Nationale
109 Sécurité du Médicament ID_RCB:2014-A01275-42).

110 1530 drugs samples, including 235 captopril and 305 amlodipine drug samples, were collected
111 as per the Guidelines for Field Surveys of the Quality of Medicines¹². 10 countries were
112 concerned: Benin, Burkina Faso, Congo, Democratic Republic of the Congo, Guinea, Côte
113 d'Ivoire, Mauritania, Niger, Senegal and Togo. In these countries, samples were obtained from
114 licensed (pharmacy) sources selected randomly and unlicensed (street market) places of sale
115 chosen as per the local investigator's convenience. Drug samples were purchased in the
116 capital city and when possible in one city located close to the country's border. Medicines
117 were obtained by the study investigator's staff who posed as customers. The pharmacies were
118 randomly chosen from a list provided by the Council of the Order of Pharmacists of each
119 country. Unlicensed markets were identified based on the local investigator's knowledge. For
120 each drug sample, investigators were asked to collect generic versions and brand name
121 versions of the drug if available. After purchase, all drugs were stored at ambient temperature,
122 in a dry place avoiding direct sunlight. Samples were sent via courier to the coordinating centre
123 in France.

124 2.2. Reagents and sample preparation

125 Amlodipine besylate, captopril, iodine, sodium thiosulfate and triethylamine (>99% pure)
126 were purchased from Sigma Aldrich (St. Quentin Fallavier, France). Analytical grade
127 acetonitrile and methanol came from VWR Prolabo®Chemicals (Fontenay-sous-Bois, France).
128 Ultrapure water was produced by the Q-Pod Milli-Q® system (Millipore, Molsheim, France).
129 The stationary phase consisted of a Kinetex®(Phenomenex, Torrance, U.S.A.) C18 column (4.6
130 mmx250 mm, i.d., 5 µm).
131 Standard solutions were prepared as per the recommendations provided in the United States
132 Pharmacopeia (USP) monograph of amlodipine besylate and captopril tablets. Captopril
133 disulfide was obtained by using the protocol proposed in the European Pharmacopeia (Ph.
134 Eur.) monograph of captopril.

135 2.3 Analytical conditions

136 Chemical analyses of samples were achieved by the Department of Laboratories in Paris
137 (AGEPS, AP-HP). Chromatographic analyses were performed using a Dionex Ultimate 3000
138 system (DIONEX, Ulis, France) coupled to a diode array detector. Chromatographic conditions
139 as well as suitability tests were performed as per the recommendations provided in the
140 monograph of amlodipine tablet (USP) and captopril (Ph. Eur.).

141 Identification of blisters was achieved using Fourier Transformed Infrared Spectroscopy (FTIR)
142 FTIR Perkin-Elmer Spectrum 2000® spectrometer (Villebon-sur-Yvette, France) with a diamond
143 crystal. Resolution, scan range and number of accumulated scans per spectrum were set to

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3 144 0.5 cm⁻¹, 4000 to 400 cm⁻¹ and 3, respectively. Spectrum were acquired on each side of the
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5 145 blisters using attenuated total reflectance mode.
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9 146 2.4. Statistical analysis

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13 147 All statistical tests were performed using GraphPad Prism version 8.3.1. for Windows
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15 148 (GraphPad Software, La Jolla California, USA). Nonparametric Spearman's correlation
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17 149 coefficient (r) was calculated to measure the strength of the association between the peak
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19 150 surface area of captopril and that of captopril disulfide. Mann-Whitney tests were used to
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21 151 compare the median between drugs with lower and those with higher protection from
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23 152 humidity packaging blisters.
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29 153 3. Results and discussion

30 154 3.1. Collected drug samples

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34 155 A total of 305 amlodipine and 235 captopril drug samples were collected in the ten countries.
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37 156 The amount of drug samples collected as a function of the countries and their place of
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39 157 purchase is summarized in Table 1. Overall, 150 of the 305 amlodipine and 140 of the 235
40
41 158 captopril drug samples considered in the study were obtained from licensed places of sale
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43 159 (Table 1). 265 and 195 drug samples were respectively identified as amlodipine and captopril
44
45 160 generic drugs.
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47
48 161 Table 1: number of collected amlodipine and captopril drug samples as a function of country
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50 162 and place of purchase (i.e. licensed or unlicensed). DRC: Democratic Republic of the Congo;
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52 163 NA : not applicable.
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Amlodipine

Captopril

	Licensed	Unlicensed	Total	Licensed	Unlicensed	Total
Benin	20	30	50	20	30	50
Burkina Faso	10	NA	10	20	NA	20
Congo- Brassaville	NA	30	30	10	10	20
Côte d'Ivoire	30	45	75	30	15	45
DRC	20	NA	20	10	NA	10
Guinea	NA	10	10	NA	10	10
Mauritania	20	20	40	30	NA	30
Niger	30	NA	30	NA	10	10
Senegal	NA	20	20	NA	20	20
Togo	20	NA	20	20	NA	20
Total	150	155	305	140	95	235

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165 3.2. Chromatographic purity profiles of the drugs

166 3.2.1. Captopril samples

167 The qualitative study of the chromatograms of the 235 captopril drug samples showed that
 168 the 31 samples with drug amounts inferior to 90% (w/w%) contained the same impurity (Fig.
 169 1). The detected impurity was assigned to captopril disulfide as it had the same retention time
 170 than this compound.

171 Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance
 172 standard solution (black), typical substandard drug sample (blue).

173 Further, for every chromatogram, the sum of the surface of the peak areas corresponding to
 174 captopril and that of captopril disulfide led to obtain areas recoveries between 90 and 110%
 175 compared to that of the captopril reference solution. Therefore, one could infer that the lack
 176 of drug captopril may be linked to the presence of captopril disulfide. As a result, a correlation

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3 177 study between the surface of the peak of captopril and that of captopril disulfide impurity was
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5 178 carried out and yielded a negative Spearman coefficient with a value of -0.613 ($p=0.0069$).
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9 179 3.2.2. Amlodipine samples

10
11 180 Unlike captopril, the 305 chromatographic profiles obtained for amlodipine samples strongly
12
13 181 differed from one to the other, implying that the drugs did not contain the same impurities
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15 182 (Fig. 2). Indeed, under the analytical conditions recommended in the USP monograph, two
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17 183 categories of chromatographic profiles were obtained as a function of the studied samples.
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22 184 A first category of chromatographic profiles consisted of samples containing an impurity with
23
24 185 a relative retention time of about 0.5 (Fig 2. a) as compared to amlodipine, referred in the USP
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26 186 as amlodipine related compound A. In 10 chromatograms, the surface of the peak of this
27
28 187 impurity exceeded the USP acceptance criteria for this impurity ($<1.0\%$, w/w).
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33 188 The second category of chromatographic profiles comprised a peak with a relative retention
34
35 189 time of about 0.8 (Fig 2.b) corresponding to a degradation related impurity, namely an
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37 190 amlodipine-lactose interaction product. For 30 sample solutions, the area of the peak of this
38
39 191 impurity compared to that of amlodipine exceeded the USP acceptance criteria for this
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41 192 impurity ($<0.5\%$ w/w).
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47 193 Fig. 2: typical chromatographic profiles for samples containing impurity A (a) and samples
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49 194 containing amlodipine-lactose interaction product (b).
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52 195 3.3. Blisters identification

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56 196 The identity and aspect of the blisters materials strongly differed between amlodipine and
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58 197 captopril and within drug samples of the same drug.
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3 198 FTIR analysis were performed on each side of the plastic blisters in order to identify its main
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6 199 compounds. Based on the analysis, two groups could be established: those with a spectrum
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8 200 corresponding to polyvinyl-chloride's one (PVC, red and blue spectrum, Fig. 3) and those which
9
10 201 have two spectral bands (597 and 527 cm^{-1}) characteristic of the polyvinylidene-chloride
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13 202 presence¹³ (PVDC, green spectrum, Fig. 3).

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16 203 Fig. 3: typical FTIR spectrum of a polyvinyl-chloride (PVC) polymer packaging with (green
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19 204 spectrum) and without (blue and red spectrum) polyvinylidene-chloride (PVDC).

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23 205 The analysis highlighted that 105 out of the 235 captopril drug samples were packaged in
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25 206 blisters containing PVDC and 130 in blisters only composed of PVC. In the case of amlodipine,
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28 207 none of the plastic blisters used to package the 245 amlodipine samples contained PVDC
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30 208 (Table 2). 60 amlodipine samples were packaged in blisters consisting of foil-foil sealed
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33 209 aluminium (Table 2).

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35
36 210 Table 2: number of amlodipine and captopril drug samples as a function of blister identity.

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38 211 PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride.
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Blister identity	Amlodipine	Captopril
PVC only	245	130
Aluminium only	60	None
PVC-PVDC	None	105

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213 3.4. Drugs assays as a function of blister identifications

214 From the results obtained in 3.2., captopril assays results were separated based on the
215 presence of PVDC or not (PVC only). Amlodipine assay results were divided in two groups: the
216 group of samples packaged in foil-foil sealed aluminium blisters and the one packaged in PVC
217 blisters (PVC only). The results are provided in figure 4.

218 Fig.4: medians of drug assays with interquartile ranges as a function of the drug and the blister
219 composition. PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride; ns: non statistically
220 significant; ****: $p < 0.0001$.

221 For amlodipine, no statistical difference in dosage ($p = 0.7684$) was observed between the
222 group with foil-foil aluminium packaging (median=96.10, $n = 60$) and the group with PVC blister
223 packaging (median=96.60, $n = 245$).

224 For captopril, on the other hand, a statistical difference ($p < 0.0001$) was found between the
225 group of samples stored in blisters containing only PVC (median=96.60, $n = 130$) and those
226 stored in blisters containing PVC/PVDC (median=100.0, $n = 105$).

227 4. Discussion

228 Sub-standard cardiac medicines represent a serious health hazard and result in significant
229 morbidity and mortality⁴. The SEVEN study was the first major study of cardiovascular drugs
230 in Sub-Saharan Africa⁵⁻⁷. The analysis of samples collected in Africa and through various sales
231 spots of all kinds showed that among the products used in cardiovascular diseases, about 15-
232 20% were of low quality, and the prevalence of poor quality is higher for the products
233 containing amlodipine and captopril. Given this observation and the fact that these two active

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3 234 substances are particularly sensitive to humidity and heat, the question arose as to whether
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5 235 the intrinsic stability of the latter had contributed more to the quality defects highlighted at
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8 236 the time. So, in this follow-up study, we have sought to provide some answers to this question
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10 237 by focusing on the search of associated degradation products and the nature of the packaging
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13 238 used at the time of the on-site collections.

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16 239 We therefore sought to supplement the assay results with chromatographic purity studies on
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18 240 samples of the various cardiological drugs samples collected during the SEVEN study. We
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20 241 noted that the levels of impurities are particularly high for captopril and amlodipine samples
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22 242 (>0.5% compared to the considered active substance), as is their high prevalence of
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24 243 underdosing relative to other products, as previously mentioned. Of course, this problem
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26 244 could have been caused by the use of poor-quality active ingredient. But the fact that it is
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28 245 specific to these two active substances did not allow us to limit to this aspect alone. We
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30 246 therefore also turned our attention to the stability issue and to what goes hand in hand with
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32 247 this aspect. The identification of chromatographic impurities present concomitantly with the
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34 248 active substance was carried out to support our hypotheses related to the degradation of
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36 249 these compounds.

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44 250 In the case of the captopril drug samples, the compound detected (Fig. 1) in addition to
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46 251 captopril was identified as captopril disulfide, a well-known degradation product of captopril
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48 252 that is prompt to be formed under moisture and heat¹⁴. In other words, this would mean that
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50 253 the underdoses initially observed in the captopril products⁶ could be attributed to the poor
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52 254 quality of the active substance used and/or, to a larger extent, to degradation occurring either
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54 255 during manufacture and/or during improper storage. However, as it is well depicted that
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56 256 captopril is far more susceptible to degradation in the presence of some excipients than alone
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3 257 ^{15,16}, one can fathom that the presence of captopril disulfide may more likely result from
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5 258 degradation due to uncontrolled exposure. As for amlodipine, chromatographic analyses
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8 259 showed in 30 samples the presence of an impurity due to amlodipine-lactose interaction (Fig.
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10 260 2 b). Such interaction was shown to occur in time and mostly under high humidity conditions¹⁷,
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12 261 which could, to some extent, explain the poor assays observed in SEVEN study⁷.

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16 262 These results are consistent with data in the literature in that the moisture vapour
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18 263 transmission rate¹⁸ (MVTR) must be controlled as it is critical to the stability of captopril¹⁴ and
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20 264 amlodipine¹⁹. The corollary is that we have indeed shown, in the case of captopril, a significant
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22 265 difference ($p < 0.0001$, Fig. 4) in active substance content between tablets extracted from PVC
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24 266 blisters and those from PVC-PVDC blisters that exhibit lower MVTR¹⁸. The results for
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26 267 amlodipine are not statistically conclusive, but somehow the trend is noticeable since samples
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28 268 with a peak area of the amlodipine-lactose interaction product greater than 0.5% (w/w) were
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30 269 exclusively from the blisters with higher MVTR i.e. the blisters composed of PVC as opposed
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32 270 to the foil-foil aluminium ones¹⁸. Accordingly, it can be seen that the use of low moisture
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34 271 barrier packaging for these two products among the different cardiac drugs tested appears to
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36 272 have contributed to their low assay values.

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44 273 This poor quality, represented by both a lower active substance content and the presence of
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46 274 degradation products, subjects the patient to safety risks. On the one hand, any dose lower
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48 275 than the dose to which the body has developed tolerance may induce an effect that is difficult
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50 276 to predict and may even be contrary to the action of the usual dose²⁰. On the other hand, the
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52 277 degradation products formed may be toxic²¹.

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3 278 Captopril disulphide is a well-known metabolite of captopril, which can reversibly interconvert
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5 279 into captopril in the blood²². However, in human pharmacokinetic studies the amount of
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8 280 captopril disulphide in the blood never exceeds 8% of that of captopril^{22,23}. Therefore, clinical
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10 281 consequences of exposure to higher amounts of captopril disulfide are unknown. This is to be
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13 282 considered because the disulfide metabolites of sulfur-based drugs were 50 to 100 times more
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15 283 toxic on hepatocytes from isolated rats than the parent drugs²⁴.

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19 284 In some amlodipine samples, two main degradation products were detected as mentioned
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21 285 earlier, amlodipine impurity A that corresponds to the oxidation of its dihydropyridine ring
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24 286 and the amlodipine-lactose interaction product. Impurity A has been reported to be among
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26 287 the most found metabolites in urines (7%)²⁵. As its level was always inferior to 2% relative to
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29 288 the active substance in the samples analysed, one can assume that this presence may have a
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31 289 limited impact to the patients. As for the amlodipine-lactose interaction product, no data are
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34 290 currently available on its fate in the body and its toxicity, which makes it a risk to consider.

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37 291 Overall, on the basis of these additional results from the study of the chromatographic profiles
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39 292 of the collected samples, significant quantities of cardio drugs sold on African soil may not
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42 293 only present a lack of efficacy but also safety issues, due to the presence of impurities and/or
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44 294 degradation products resulting from poor storage and packaging conditions.

48 295 4.1. Limitations of the study

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52 296 This study is of an observational type and even if as much information as possible has been
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54 297 collected for the research and attribution of causes likely to be at the origin of the active
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57 298 substance dosage defect, other factors than those related to packaging and storage problems
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3 299 may be involved. The lack of active substance is most likely multi-factorial and it may be useful
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6 300 to explore with more samples when applicable.
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10 301 Conclusion

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12 302 Further studies of captopril and amlodipine samples from Africa showed that their low assays
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14 303 would result in part from the degradation of the active substance, which may indicate that
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17 304 protective measures to avoid degradation of the drug products were not commensurate with
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19 305 their unstable behaviour, particularly in the presence of moisture and heat. FTIR analyses of
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21
22 306 the samples' blisters disclosed they differ in material composition for a same active substance.
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24 307 In the case of captopril, statistical analysis has shown that the active substance content of
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27 308 products in tighter blister packs is higher than that of products in less moisture-resistant
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29 309 packaging. In the case of amlodipine, only the samples with low moisture resistant packaging
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31
32 310 had high amounts (>0.5% w/w) of degradation products.
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35 311 Using packaging with elevated moisture barrier properties could be a significant leverage to
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37 312 reduce the prevalence of substandard drugs in countries where medicines circuit and storage
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40 313 are poorly controlled. It is therefore also important to make the health authorities in these
41
42 314 countries aware that the quality of packaging must also be taken into account when evaluating
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45 315 medicines supplied.
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49 316 Figure legend/caption

50
51 317 Table 1: number of collected amlodipine and captopril drug samples as a function of country
52
53
54 318 and place of purchase (i.e. licensed or unlicensed). DRC: Democratic Republic of the Congo;
55
56 319 NA : not applicable.
57
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320 Table 2: number of amlodipine and captopril drug samples as a function of blister identity.

321 PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride.

322 Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance
323 standard solution (black), typical substandard drug sample (blue).

324 Fig. 2: typical chromatographic profiles for samples containing impurity A (a) and samples
325 containing amlodipine-lactose interaction product (b).

326 Fig. 3: typical FTIR spectrum of a polyvinyl-chloride (PVC) polymer packaging with (green
327 spectrum) and without (blue and red spectrum) polyvinylidene-chloride (PVDC).

328 Fig. 4: medians of drug assays with interquartile ranges as a function of the drug and the blister
329 composition. PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride; ns: non statistically
330 significant; ****: $p < 0.0001$.

331 Footnotes

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340 Drafting of the manuscript: all authors.

341 Final approval of the version to be published: all authors.

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2
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4

5 347 **Competing interests**
6

7 348 None.
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10 349 **Patient consent for publication**
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12 350 No patient involved (Not required).
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15 351 **Data sharing statement**
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17 352 No additional data available.
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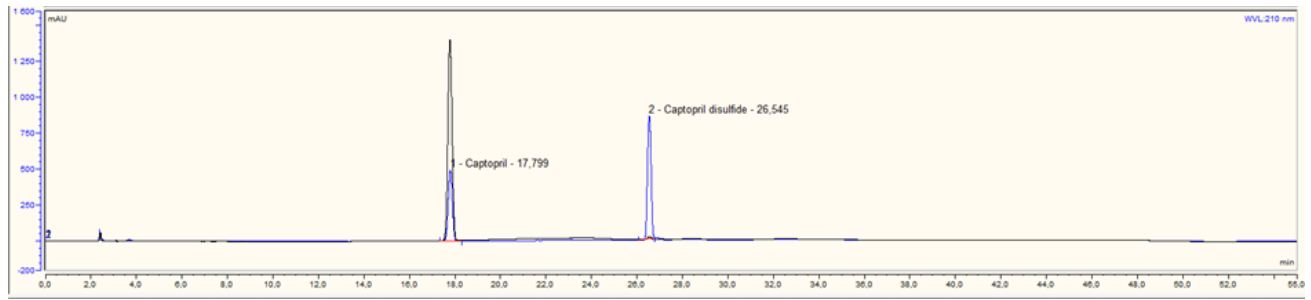


Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance standard solution (black), typical substandard drug sample (blue).

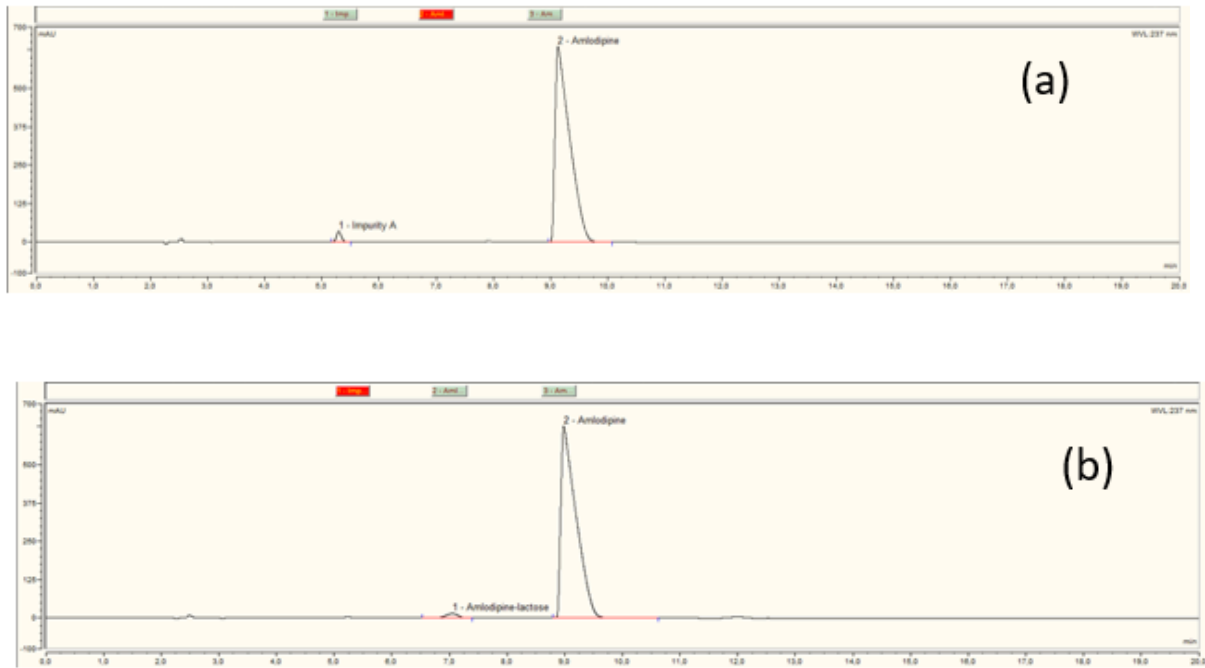


Fig. 2 : typical chromatographic profiles for samples containing impurity A (a) and samples containing amlodipine-lactose interaction product (b)

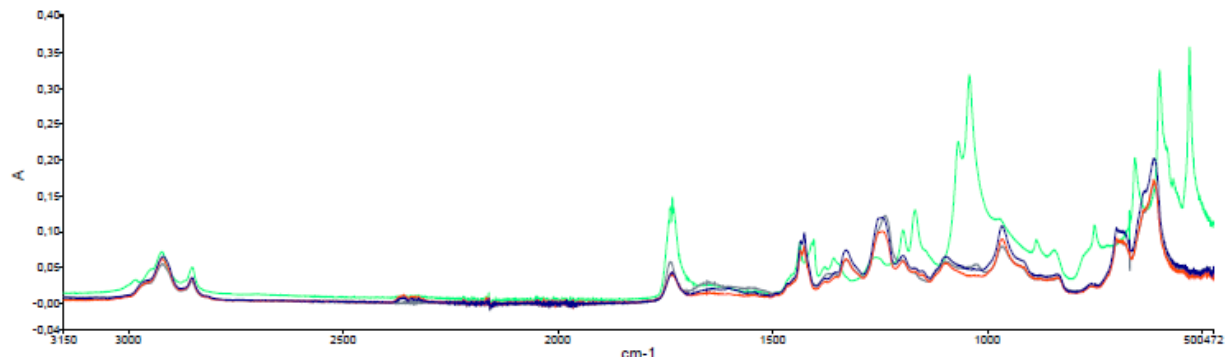


Fig. 3 : typical FTIR spectrum of a polyvinyl-chloride (PVC) polymer packaging with (green spectrum) and without (blue and red spectrum) polyvinylidene-chloride (PVDC).

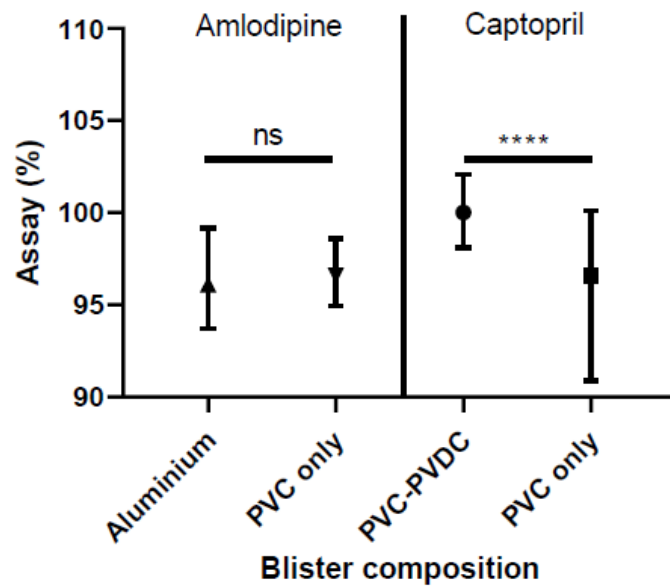


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A post hoc study to investigate the potential causes of poor quality of cardiovascular medicines collected in sub-Saharan countries

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5 1 A post hoc study to investigate the potential causes of poor
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9 2 quality of cardiovascular medicines collected in sub-Saharan
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14 3 countries
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23 Abstract

24 Objectives

25 The incidence of cardiovascular diseases is increasing and there is a growing need to provide
26 access to quality cardio drugs in Africa. In the SEVEN study, we analysed 1530 cardiovascular
27 drug samples randomly collected from ten African countries. By that time, of the seven drugs
28 products analysed, only those containing amlodipine and captopril had very low assay values
29 with active substance contents that could be less than 75% of those expected. In this article
30 we investigate complementary aspects of the amlodipine and captopril samples so to explain
31 the previously observed low assays for these two drugs.

32 Design

33 Post-hoc analysis of the captopril and amlodipine drugs samples and their packages collected
34 in the context of the SEVEN study.

35 Setting

36 10 countries were concerned: Benin, Burkina Faso, Congo, Democratic Republic of the Congo,
37 Guinea, Côte d'Ivoire, Mauritania, Niger, Senegal and Togo.

38 Participants

39 Local scientists and hospital practitioners collected the drug samples in the 10 African
40 countries.

41 Outcome measures

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3 42 The drug amount and the relative amounts of drug impurities as well as the main compounds
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5 43 of the drugs packaging were analysed.
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9 44 Results

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12 45 Identification of the blister packaging of the samples led to separate both amlodipine and
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14 46 captopril drug samples in two groups. Mann Whitney's bilateral test showed a significant
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16 47 difference ($p < 0.0001$) between the median value of the captopril dosage when tablets are
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18 48 packaged in blisters providing higher protection to humidity ($n=105$) as opposed to the tablets
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20 49 packaged in blisters providing lower humidity protection ($n=130$).
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26 50 Conclusion

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29 51 Based on these results, particular attention should be paid to the materials and types of
30
31 52 packaging used in order to minimize the lack of control over the exposures and drug circuits
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33 53 present in these different countries.
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39 54 Article summary : strengths and limitations of this study

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43 55 • Captopril and amlodipine-based drug samples were randomly collected in licensed and
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45 56 unlicensed places of sale in Africa to be analysed
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48 57 • The countries involved in this study were Benin, Burkina Faso, Congo, Democratic
49
50 58 Republic of the Congo, Guinea, Côte d'Ivoire, Mauritania, Niger, Senegal and Togo
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53 59 • Assay and impurity profile were performed for quality and stability assessment
54
55 60 considering, where applicable, the type of primary packaging used
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61 1. Introduction

62 Substandard drugs generally pose a serious health concern from several perspectives and this
63 is particularly prevalent in developing countries where control regulations are poorly
64 developed. Numerous cases of quality defects have been reported^{1,2}, mostly in connection
65 with anti-infective drugs³. However, since cardiovascular diseases are currently the most
66 important non-communicable diseases in most low- and middle-income countries, it is critical
67 to ensure that these diseases are addressed in a comprehensive manner⁴. Thus, under the
68 initiative of X. Jouven, the study named SEVEN has been set up with the aim of evaluating the
69 quality of the cardiovascular drugs present in ten African countries⁵⁻⁷, by exploring the case
70 of 7 commonly used cardiac drugs, namely acenocoumarol, simvastatin, furosemide,
71 hydrochlorothiazide, captopril, atenolol and amlodipine. It was showed that branded drugs
72 were less likely to be of poor quality compared to generic or medicines with unknown version
73 ($p < 0.001$) ; conversely place of sale was not significantly associated with the proportion of
74 poor quality ($p = 0.29$)⁶. Examination of the identity and assay of the active substances in the
75 various samples collected revealed that among the products tested, only those based on
76 amlodipine and captopril had very low active substance content that could be less than 75%
77 of those expected⁶. The observation that the biggest quality defects were highlighted and
78 circumscribed around these two molecules motivated the team to search for additional
79 information in particular regarding their stability.

80 For drugs where the quality defect is unintentional, according to Johnston et al, low dosage
81 levels of active substance may be the result of a variety of factors, including inadequate
82 package design or quality². Yet, despite the fact that the influence of packaging changes on

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3 83 the stability of medicinal products is now well established^{8,9}, to the best of our knowledge,
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5 84 the studies of the quality of real field samples have so far focused only on dosage units without
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8 85 taking into account the type of packaging used or their chemical composition³. However, the
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10 86 concept of packaging is equally important to assess, especially since the drug products need
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13 87 to be kept stable in difficult climatic conditions, where, for example, residual moisture can
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15 88 reach 88% in sub-Saharan Africa¹⁰ and the ability of a plastic blister pack to protect a drug
16
17 89 from moisture is highly dependent on its design and composition¹¹.

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21 90 It is in this context that this study was taking place considering samples of captopril and
22
23 91 amlodipine products, collected as part of the SEVEN study⁵⁻⁷, as tracer products. On these
24
25 92 various samples was carried out the search for degradation products and/or chromatographic
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27 93 related substances as well as the identification of the packaging actually present on the
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29 94 products collected in several African countries, by trying, where possible and relevant, to
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31 95 reconcile the different data and make them meaningful in relation to the potential causes of
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33 96 underdosing previously highlighted for these two drugs.
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40 97 2. Material and method

45 98 2.1. Sample collection

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49 99 The methodology and design of the samples collection are described in detail in the articles
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51 100 published in the context of the SEVEN study⁵⁻⁷. A multidisciplinary collaborative team of
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53 101 epidemiologists, cardiologists and pharmacists from France and Africa conceived and
54
55 102 designed the study. It was registered with the French national drug agency (Agence Nationale
56
57 103 Sécurité du Médicament ID_RCB:2014-A01275-42).
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3 104 1530 drugs samples, including 235 captopril and 305 amlodipine drug samples, were collected
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6 105 as per the Guidelines for Field Surveys of the Quality of Medicines¹². 10 countries were
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8 106 concerned: Benin, Burkina Faso, Congo, Democratic Republic of the Congo, Guinea, Côte
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10 107 d'Ivoire, Mauritania, Niger, Senegal and Togo. In these countries, samples were obtained from
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12 108 licensed (pharmacy) sources selected randomly and unlicensed (street market) places of sale
13
14
15 109 chosen as per the local investigator's convenience. Drug samples were purchased in the
16
17
18 110 capital city and when possible in one city located close to the country's border. Medicines
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20 111 were obtained by the study investigator's staff who posed as customers. The pharmacies were
21
22 112 randomly chosen from a list provided by the Council of the Order of Pharmacists of each
23
24 113 country. Unlicensed markets were identified based on the local investigator's knowledge. For
25
26 114 each drug sample, investigators were asked to collect generic versions and brand name
27
28 115 versions of the drug if available. After purchase, all drugs were stored at ambient temperature,
29
30 116 in a dry place avoiding direct sunlight. Samples were sent via courier to the coordinating centre
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32
33
34 117 in France.

38 39 118 2.2. Reagents and sample preparation

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42 119 Amlodipine besylate, captopril, iodine, sodium thiosulfate and triethylamine (>99% pure)
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44 120 were purchased from Sigma Aldrich (St. Quentin Fallavier, France). Analytical grade
45
46 121 acetonitrile and methanol came from VWR Prolabo®Chemicals (Fontenay-sous-Bois, France).
47
48 122 Ultrapure water was produced by the Q-Pod Milli-Q® system (Millipore, Molsheim, France).
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50 123 The stationary phase consisted of a Kinetex®(Phenomenex, Torrance, U.S.A.) C18 column (4.6
51
52 124 mmx250 mm, i.d., 5 µm).

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3 125 Standard solutions were prepared as per the recommendations provided in the United States
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5 126 Pharmacopeia (USP) monograph of amlodipine besylate and captopril tablets. Captopril
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8 127 disulfide was obtained by using the protocol proposed in the European Pharmacopeia (Ph.
9
10 128 Eur.) monograph of captopril.
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14 129 2.3 Analytical conditions

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18 130 Chemical analyses of samples were achieved by the Department of Laboratories in Paris
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20 131 (AGEPS, AP-HP). Chromatographic analyses were performed using a Dionex Ultimate 3000
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22 132 system (DIONEX, Ulis, France) coupled to a diode array detector. Chromatographic conditions
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24 133 as well as suitability tests were performed as per the recommendations provided in the
25
26 134 monograph of amlodipine tablet (USP) and captopril (Ph. Eur.).
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31 135 Identification of blisters was achieved using Fourier Transformed Infrared Spectroscopy (FTIR)
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33 136 FTIR Perkin-Elmer Spectrum 2000® spectrometer (Villebon-sur-Yvette, France) with a diamond
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35 137 crystal. Resolution, scan range and number of accumulated scans per spectrum were set to
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37 138 0.5 cm⁻¹, 4000 to 400 cm⁻¹ and 3, respectively. Spectrum were acquired on each side of the
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39 139 blisters using attenuated total reflectance mode.
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45 140 2.4. Statistical analysis

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49 141 All statistical tests were performed using GraphPad Prism version 8.3.1. for Windows
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51 142 (GraphPad Software, La Jolla California, USA). Nonparametric Spearman's correlation
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53 143 coefficient (r) was calculated to measure the strength of the association between the peak
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55 144 surface area of captopril and that of captopril disulfide. Mann-Whitney tests were used to
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3 145 compare the median between drugs with lower and those with higher protection from
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6 146 humidity packaging blisters.
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10 147 3. Results

14 148 3.1. Collected drug samples

17 149 A total of 305 amlodipine and 235 captopril drug samples were collected in the ten countries.

20 150 The amount of drug samples collected as a function of the countries and their place of
21
22 151 purchase is summarized in Table 1. Overall, 150 of the 305 amlodipine and 140 of the 235
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24 152 captopril drug samples considered in the study were obtained from licensed places of sale
25
26 153 (Table 1). 265 and 195 drug samples were respectively identified as amlodipine and captopril
27
28 154 generic drugs.
29
30

32 155 Table 1: number of collected amlodipine and captopril drug samples as a function of country
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34 156 and place of purchase (i.e. licensed or unlicensed). DRC: Democratic Republic of the Congo;
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36 157 NA : not applicable.
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	Amlodipine			Captopril		
	Licensed	Unlicensed	Total	Licensed	Unlicensed	Total
Benin	20	30	50	20	30	50
Burkina Faso	10	NA	10	20	NA	20
Congo- Brassaville	NA	30	30	10	10	20
Côte d'Ivoire	30	45	75	30	15	45
DRC	20	NA	20	10	NA	10
Guinea	NA	10	10	NA	10	10
Mauritania	20	20	40	30	NA	30
Niger	30	NA	30	NA	10	10
Senegal	NA	20	20	NA	20	20
Togo	20	NA	20	20	NA	20
Total	150	155	305	140	95	235

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159 3.2. Chromatographic purity profiles of the drugs

160 3.2.1. Captopril samples

161 The qualitative study of the chromatograms of the 235 captopril drug samples showed that
162 the 31 samples with drug amounts inferior to 90% (w/w%) contained the same impurity (Fig.
163 1). The detected impurity was assigned to captopril disulfide as it had the same retention time
164 than this compound.

165 Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance
166 standard solution (black), typical substandard drug sample (blue).

167 Further, for every chromatogram, the sum of the surface of the peak areas corresponding to
168 captopril and that of captopril disulfide led to obtain areas recoveries between 90 and 110%
169 compared to that of the captopril reference solution. Therefore, one could infer that the lack
170 of drug captopril may be linked to the presence of captopril disulfide. As a result, a correlation
171 study between the surface of the peak of captopril and that of captopril disulfide impurity was
172 carried out and yielded a negative Spearman coefficient with a value of -0.613 ($p=0.0069$).

173 3.2.2. Amlodipine samples

174 Unlike captopril, the 305 chromatographic profiles obtained for amlodipine samples strongly
175 differed from one to the other, implying that the drugs did not contain the same impurities
176 (Fig. 2). Indeed, under the analytical conditions recommended in the USP monograph, two
177 categories of chromatographic profiles were obtained as a function of the studied samples.

178 A first category of chromatographic profiles consisted of samples containing an impurity with
179 a relative retention time of about 0.5 (Fig 2. a) as compared to amlodipine, referred in the USP

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3 180 as amlodipine related compound A. In 10 chromatograms, the surface of the peak of this
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5 181 impurity exceeded the USP acceptance criteria for this impurity (<1.0 %, w/w).

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9 182 The second category of chromatographic profiles comprised a peak with a relative retention
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11 183 time of about 0.8 (Fig 2.b) corresponding to a degradation related impurity, namely an
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13 184 amlodipine-lactose interaction product. For 30 sample solutions, the area of the peak of this
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15 185 impurity compared to that of amlodipine exceeded the USP acceptance criteria for this
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17 186 impurity (<0.5% w/w).

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22 187 Fig. 2: typical chromatographic profiles for samples containing impurity A (a) and samples
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24 188 containing amlodipine-lactose interaction product (b).

25 26 27 28 189 3.3. Blisters identification

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32 190 The identity and aspect of the blisters materials strongly differed between amlodipine and
33
34 191 captopril and within drug samples of the same drug.

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38 192 FTIR analysis were performed on each side of the plastic blisters in order to identify its main
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40 193 compounds. Based on the analysis, two groups could be established: those with a spectrum
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42 194 corresponding to polyvinyl-chloride's one (PVC, red and blue spectrum, Fig. 3) and those which
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44 195 have two spectral bands (597 and 527 cm^{-1}) characteristic of the polyvinylidene-chloride
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46 196 presence¹³ (PVDC, green spectrum, Fig. 3).

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51 197 Fig. 3: typical FTIR spectrum of a polyvinyl-chloride (PVC) polymer packaging with (green
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53 198 spectrum) and without (blue and red spectrum) polyvinylidene-chloride (PVDC).

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55
56
57 199 The analysis highlighted that 105 out of the 235 captopril drug samples were packaged in
58
59 200 blisters containing PVDC and 130 in blisters only composed of PVC. In the case of amlodipine,

201 none of the plastic blisters used to package the 245 amlodipine samples contained PVDC
 202 (Table 2). 60 amlodipine samples were packaged in blisters consisting of foil-foil sealed
 203 aluminium (Table 2).

204 Table 2: number of amlodipine and captopril drug samples as a function of blister identity.
 205 PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride.

Blister identity	Amlodipine	Captopril
PVC only	245	130
Aluminium only	60	None
PVC-PVDC	None	105

207 3.4. Drugs assays as a function of blister identifications

208 From the results obtained in 3.2., captopril assays results were separated based on the
 209 presence of PVDC or not (PVC only). Amlodipine assay results were divided in two groups: the
 210 group of samples packaged in foil-foil sealed aluminium blisters and the one packaged in PVC
 211 blisters (PVC only). The results are provided in figure 4.

212 Fig.4: medians of drug assays with interquartile ranges as a function of the drug and the blister
 213 composition. PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride; ns: non statistically
 214 significant; ****: $p < 0.0001$.

215 For amlodipine, no statistical difference in dosage ($p = 0.7684$) was observed between the
 216 group with foil-foil aluminium packaging (median=96.10, $n = 60$) and the group with PVC blister
 217 packaging (median=96.60, $n = 245$).

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3 218 For captopril, on the other hand, a statistical difference ($p < 0.0001$) was found between the
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5 219 group of samples stored in blisters containing only PVC (median=96.60, n=130) and those
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8 220 stored in blisters containing PVC/PVDC (median=100.0, n=105).
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11 221 4. Discussion

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16 222 Sub-standard cardiac medicines represent a serious health hazard and result in significant
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18 223 morbidity and mortality⁴. The SEVEN study was the first major study of cardiovascular drugs
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20 224 in Sub-Saharan Africa⁵⁻⁷. The analysis of samples collected in Africa and through various sales
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22 225 spots of all kinds showed that among the products used in cardiovascular diseases, about 15-
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24 226 20% were of low quality, and the prevalence of poor quality is higher for the products
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26 227 containing amlodipine and captopril. Given this observation and the fact that these two active
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28 228 substances are particularly sensitive to humidity and heat, the question arose as to whether
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30 229 the intrinsic stability of the latter had contributed more to the quality defects highlighted at
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32 230 the time. So, in this follow-up study, we have sought to provide some answers to this question
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34 231 by focusing on the search of associated degradation products and the nature of the packaging
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36 232 used at the time of the on-site collections.
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45 233 We therefore sought to supplement the assay results with chromatographic purity studies on
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47 234 samples of the various cardiological drugs samples collected during the SEVEN study. We
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49 235 noted that the levels of impurities are particularly high for captopril and amlodipine samples
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51 236 ($>0.5\%$ compared to the considered active substance), as is their high prevalence of
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53 237 underdosing relative to other products, as previously mentioned. Of course, this problem
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55 238 could have been caused by the use of poor-quality active ingredient. But the fact that it is
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57 239 specific to these two active substances did not allow us to limit to this aspect alone. We
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1
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3 240 therefore also turned our attention to the stability issue and to what goes hand in hand with
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5 241 this aspect. The identification of chromatographic impurities present concomitantly with the
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8 242 active substance was carried out to support our hypotheses related to the degradation of
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10 243 these compounds.

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14 244 In the case of the captopril drug samples, the compound detected (Fig. 1) in addition to
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16 245 captopril was identified as captopril disulfide, a well-known degradation product of captopril
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18 246 that is prompt to be formed under moisture and heat¹⁴. In other words, this would mean that
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21 247 the underdoses initially observed in the captopril products⁶ could be attributed to the poor
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23 248 quality of the active substance used and/or, to a larger extent, to degradation occurring either
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26 249 during manufacture and/or during improper storage. However, as it is well depicted that
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29 250 captopril is far more susceptible to degradation in the presence of some excipients than alone
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31 251 ^{15,16}, one can fathom that the presence of captopril disulfide may more likely result from
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33 252 degradation due to uncontrolled exposure. As for amlodipine, chromatographic analyses
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36 253 showed in 30 samples the presence of an impurity due to amlodipine-lactose interaction (Fig.
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38 254 2 b). Such interaction was shown to occur in time and mostly under high humidity conditions¹⁷,
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41 255 which could, to some extent, explain the poor assays observed in SEVEN study⁷.

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43
44 256 These results are consistent with data in the literature in that the moisture vapour
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46 257 transmission rate¹⁸ (MVTR) must be controlled as it is critical to the stability of captopril¹⁴ and
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48
49 258 amlodipine¹⁹. The corollary is that we have indeed shown, in the case of captopril, a significant
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52 259 difference ($p < 0.0001$, Fig. 4) in active substance content between tablets extracted from PVC
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54 260 blisters and those from PVC-PVDC blisters that exhibit lower MVTR¹⁸. The results for
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57 261 amlodipine are not statistically conclusive, but somehow the trend is noticeable since samples
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59 262 with a peak area of the amlodipine-lactose interaction product greater than 0.5% (w/w) were
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3 263 exclusively from the blisters with higher MVTR i.e. the blisters composed of PVC as opposed
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5 264 to the foil-foil aluminium ones¹⁸. Accordingly, it can be seen that the use of low moisture
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8 265 barrier packaging for these two products among the different cardiac drugs tested appears to
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10 266 have contributed to their low assay values.

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14 267 This poor quality, represented by both a lower active substance content and the presence of
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16 268 degradation products, subjects the patient to safety risks. On the one hand, any dose lower
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18 269 than the dose to which the body has developed tolerance may induce an effect that is difficult
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20 270 to predict and may even be contrary to the action of the usual dose²⁰. On the other hand, the
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22 271 degradation products formed may be toxic²¹.

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27 272 Captopril disulphide is a well-known metabolite of captopril, which can reversibly interconvert
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29 273 into captopril in the blood²². However, in human pharmacokinetic studies the amount of
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31 274 captopril disulphide in the blood never exceeds 8% of that of captopril^{22,23}. Therefore, clinical
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33 275 consequences of exposure to higher amounts of captopril disulfide are unknown. This is to be
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35 276 considered because the disulfide metabolites of sulfur-based drugs were 50 to 100 times more
36
37 277 toxic on hepatocytes from isolated rats than the parent drugs²⁴.

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43 278 In some amlodipine samples, two main degradation products were detected as mentioned
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45 279 earlier, amlodipine impurity A that corresponds to the oxidation of its dihydropyridine ring
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47 280 and the amlodipine-lactose interaction product. Impurity A has been reported to be among
48
49 281 the most found metabolites in urines (7%)²⁵. As its level was always inferior to 2% relative to
50
51 282 the active substance in the samples analysed, one can assume that this presence may have a
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53 283 limited impact to the patients. As for the amlodipine-lactose interaction product, no data are
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55 284 currently available on its fate in the body and its toxicity, which makes it a risk to consider.
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3 285 Overall, on the basis of these additional results from the study of the chromatographic profiles
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5 286 of the collected samples, significant quantities of cardio drugs sold on African soil may not
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7
8 287 only present a lack of efficacy but also safety issues, due to the presence of impurities and/or
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10 288 degradation products resulting from poor storage and packaging conditions.
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14 289 4.1. Limitations of the study

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18 290 This study is of an observational type and even if as much information as possible has been
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20 291 collected for the research and attribution of causes likely to be at the origin of the active
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23 292 substance dosage defect, other factors than those related to packaging and storage problems
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25 293 may be involved. The lack of active substance is most likely multi-factorial and it may be useful
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28 294 to explore with more samples when applicable.
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31 295 Conclusion

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34 296 Further studies of captopril and amlodipine samples from Africa showed that their low assays
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36 297 would result in part from the degradation of the active substance, which may indicate that
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39 298 protective measures to avoid degradation of the drug products were not commensurate with
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41
42 299 their unstable behaviour, particularly in the presence of moisture and heat. FTIR analyses of
43
44 300 the samples' blisters disclosed they differ in material composition for a same active substance.
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46
47 301 In the case of captopril, statistical analysis has shown that the active substance content of
48
49 302 products in tighter blister packs is higher than that of products in less moisture-resistant
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51 303 packaging. In the case of amlodipine, only the samples with low moisture resistant packaging
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53
54 304 had high amounts (>0.5% w/w) of degradation products.
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57 305 Using packaging with elevated moisture barrier properties could be a significant leverage to
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60 306 reduce the prevalence of substandard drugs in countries where medicines circuit and storage

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3 307 are poorly controlled. It is therefore also important to make the health authorities in these
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5 308 countries aware that the quality of packaging must also be taken into account when evaluating
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8 309 medicines supplied.
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11 310 Figure legend/caption

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14 311 Table 1: number of collected amlodipine and captopril drug samples as a function of country
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16 312 and place of purchase (i.e. licensed or unlicensed). DRC: Democratic Republic of the Congo;
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19 313 NA : not applicable.
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23 314 Table 2: number of amlodipine and captopril drug samples as a function of blister identity.
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25 315 PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride.
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29 316 Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance
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31 317 standard solution (black), typical substandard drug sample (blue).
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35 318 Fig. 2: typical chromatographic profiles for samples containing impurity A (a) and samples
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37 319 containing amlodipine-lactose interaction product (b).
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41 320 Fig. 3: typical FTIR spectrum of a polyvinyl-chloride (PVC) polymer packaging with (green
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43 321 spectrum) and without (blue and red spectrum) polyvinylidene-chloride (PVDC).
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47 322 Fig. 4: medians of drug assays with interquartile ranges as a function of the drug and the blister
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49 323 composition. PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride; ns: non statistically
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51 324 significant; ****: $p < 0.0001$.
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53 325 Footnotes

54 326 Contributors

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334 Drafting of the manuscript: all authors.

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341

342 Competing interests

343 None declared.

344 Patient and public involvement

345 Patients and/or the public were not involved in the design, conduct, reporting or
346 dissemination of this research.

347 Patient consent for publication

348 Not required.

349 Data sharing statement

350 No additional data available.

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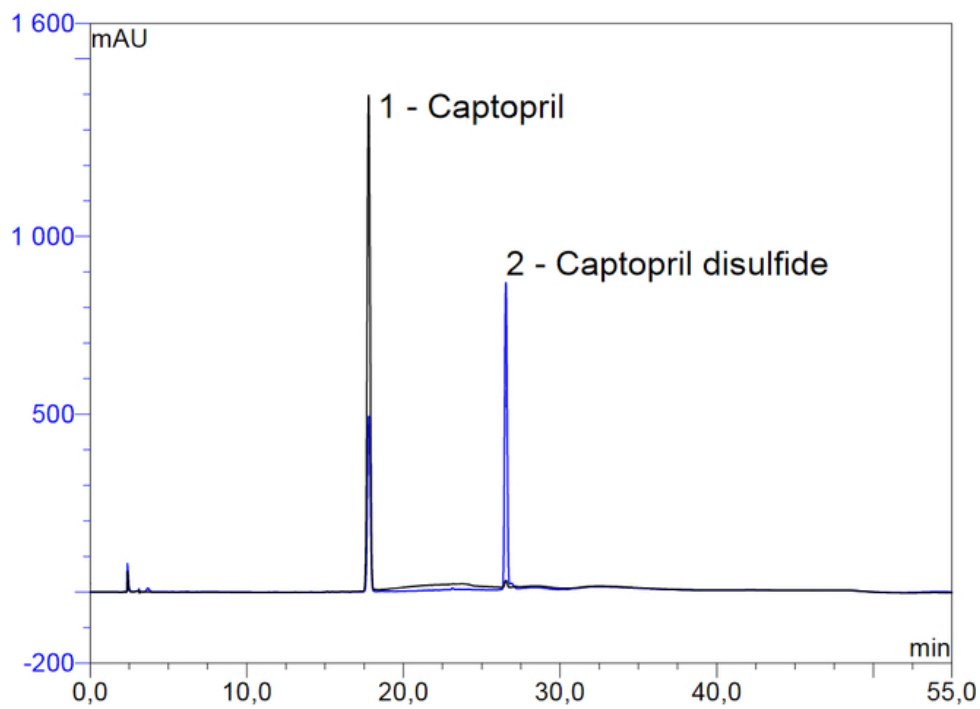


Fig. 1: typical chromatographic profiles of captopril solutions: reference active substance standard solution (black), typical substandard drug sample (blue).

57x42mm (300 x 300 DPI)

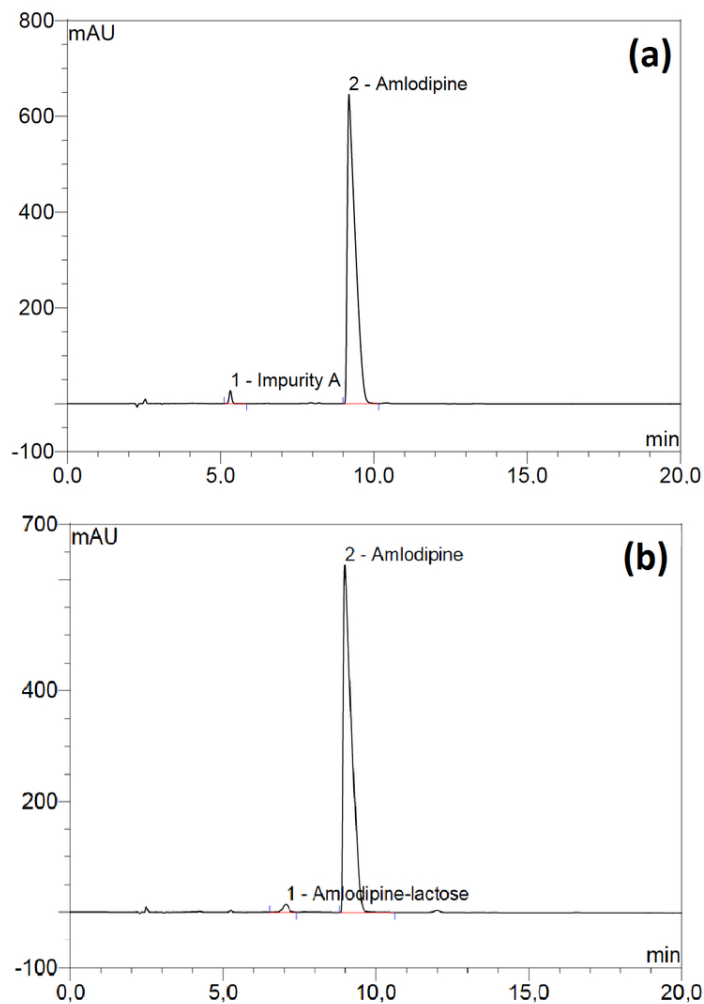


Fig. 2: typical chromatographic profiles for samples containing impurity A (a) and samples containing amlodipine-lactose interaction product (b).

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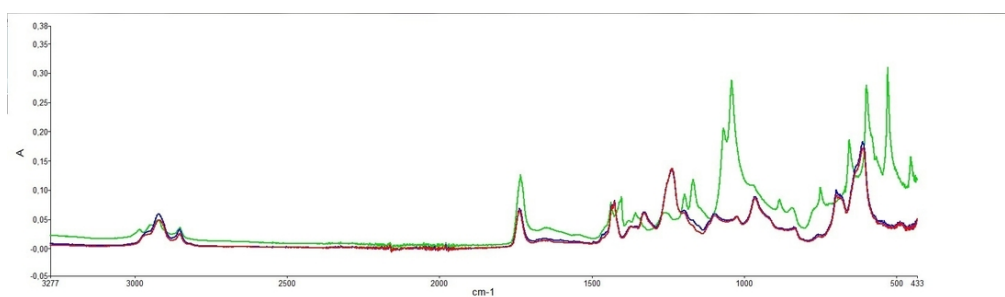


Fig. 3: typical FTIR spectrum of a polyvinyl-chloride (PVC) polymer packaging with (green spectrum) and without (blue and red spectrum) polyvinylidene-chloride (PVDC).

92x37mm (300 x 300 DPI)

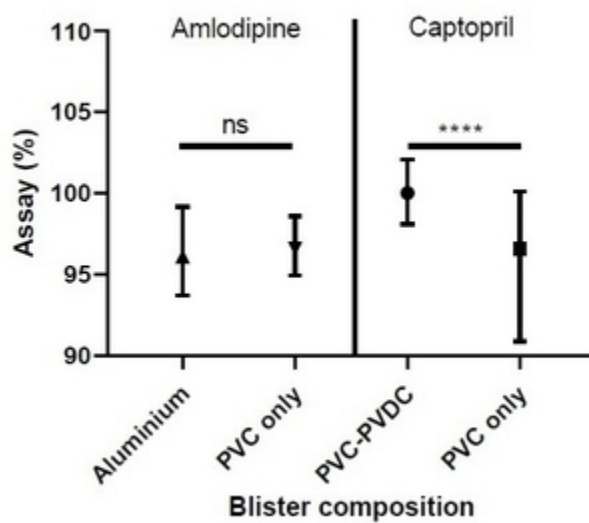


Fig.4: medians of drug assays with interquartile ranges as a function of the drug and the blister composition. PVC: polyvinyl-chloride; PVDC: polyvinylidene-chloride; ns: non statistically significant; ****: $p < 0.0001$.

27x24mm (300 x 300 DPI)