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Identification of Falsified Chloroquine Tablets in Africa at the Time of the COVID-19 Pandemic

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Abstract. Reports that chloroquine and hydroxychloroquine may be effective against COVID-19 have received worldwide attention, increasing the risk of the introduction of falsified versions of these medicines. Five different types of falsified chloroquine tablets were discovered between March 31, 2020 and April 4, 2020, in Cameroon and the Democratic Republic of Congo by locally conducted thin layer chromatographic analysis. Subsequent investigation by liquid chromatography and mass spectrometry in Germany proved the absence of detectable amounts of chloroquine and the presence of undeclared active pharmaceutical ingredients, that is, paracetamol and metronidazole, in four of the samples. The fifth sample contained chloroquine, but only 22% of the declared amount. Such products represent a serious risk to patients. Their occurrence exemplifies that once medicines or vaccines against COVID-19 may be developed, falsified products will enter the market immediately, especially in low- and middle-income countries (LMICs). Timely preparations for the detection of such products are required, including the establishment of appropriate screening technologies in LMICs.

In February 2020 and March 2020, reports that chloroquine (CQ) and hydroxychloroquine (HCQ) may be effective against COVID-19[1–4] received massive political and media attention worldwide, despite limited evidence.[5,6] Concerns have been raised that the premature off-label use of CQ and HCQ in COVID-19 may result in shortages of these medicines in their established, approved indications (i.e., against autoimmune diseases and, in case of CQ, *Plasmodium vivax* malaria).[7,8] The demand for CQ and HCQ quickly outstripped the supply, exacerbating the risk of falsified medicines entering the market.[8] We here report the recent occurrence of falsified CQ, detected in Cameroon and the Democratic Republic (DR) of Congo.

The Ecumenical Pharmaceutical Network (EPN), among other tasks, monitors medicine quality using the Global Pharma Health Fund (GPHF) Minilab,[9] a screening methodology based on thin layer chromatography (TLC) which is easy to conduct in resource-limited environments.[10] In March 2020, local member organizations of the EPN reported that both in private pharmacies and in informal markets, several types of falsified CQ tablets were appearing which, in local GPHF Minilab analysis,[11] were found not to contain CQ. Through the German Institute for Medical Mission (Difaem), the member organization of EPN which coordinates the Minilab network, the WHO Rapid Alert System, was informed, and the WHO published an international Medical Product Alert about falsified CQ tablets.[12]

In the following days, further falsified CQ samples were identified in Cameroon. Five samples were forwarded by commercial courier from Cameroon and the DR Congo to Tuebingen University, Germany. They are depicted in Figure 1, together with photos of their TLC analysis, according to the GPHF Minilab procedure.[11] Details of the samples are listed in Table 1.

Thin layer chromatography readily showed the presence of CQ in the reference solutions, visible both under UV light and in subsequent detection with iodine vapor. By contrast, CQ was not detectable in four of the investigated samples. The fifth sample showed a spot of CQ, but the compound was apparently present only in a low amount (Figure 1, sample I). For samples II and III (Figure 1), TLC analysis with UV detection showed the presence of further, undeclared compounds with a higher retention factor than CQ. The undeclared compound in sample II was also detectable by iodine staining, but the compound in sample III was not (Figure 1), indicating that these two compounds were chemically different.

These observations were confirmed at Tübingen University by high-performance liquid chromatography (HPLC) according to the U.S. Pharmacopeia.[13] As shown in Figure 2, no CQ was detected in four of the samples. By contrast, in sample I, CQ was present in an amount corresponding to 21.7 mg CQ phosphate, that is, only 21.7% of the amount stated on the label. Samples II and V showed an unknown compound with a retention time of 4.7 minutes in HPLC, and samples III, IV, and V showed a further unknown compound with a retention time of 4.5 minutes.

Liquid chromatography (LC) coupled with high-resolution mass spectrometry (HR-MS) showed that the two unknown compounds had exact molecular masses of 152.0709 and 172.0719, consistent with the masses of paracetamol and of metronidazole, respectively. Their identity was confirmed in comparison with authentic reference compounds of paracetamol and of metronidazole, showing identical retention times, molecular masses, and mass spectrometric fragmentation as the references (Supplemental Table S4, Supplemental Figures S2 and S3, Supplemental Information). The quantities of these compounds were determined as 35.7 mg paracetamol per tablet for sample II and as 126.5 mg metronidazole per tablet for sample III. Samples IV and V were found to contain smaller amounts of metronidazole, that is, 14.1 mg and 14.6 mg per tablet, respectively. Sample V additionally contained traces of paracetamol (1.6 mg per tablet).

The labeling of the five samples showed mistakes and spelling errors (Table 1), suggesting that they were produced not by established manufacturers but by criminals. The stated manufacturer of sample III, Dawa Limited, Kenya, was contacted by the local partners in the DR Congo and confirmed

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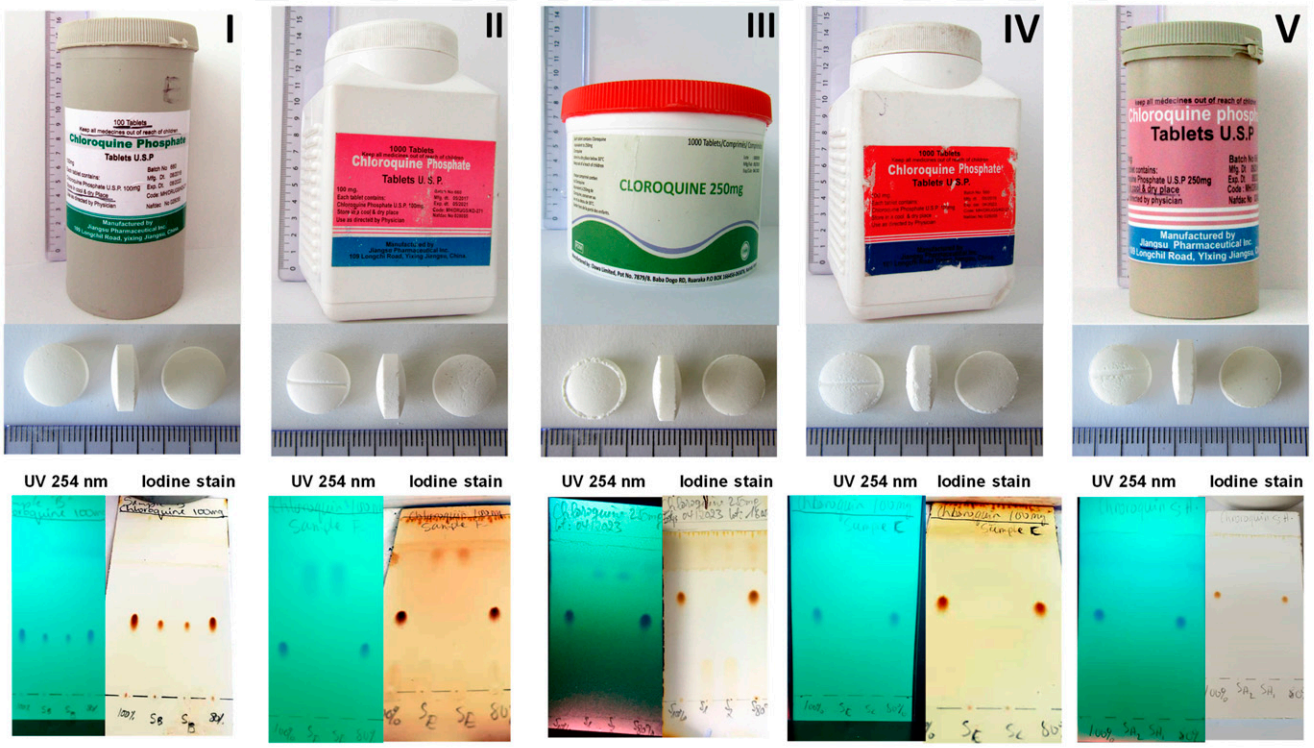

Chloroquine (CQ) amount declared:

100 mg CQ phosphate	100 mg CQ phosphate	250 mg CQ	100 mg CQ phosphate	250 mg CQ phosphate

Active principles detected:

21.7 mg CQ phosphate	no CQ 35.7 mg paracetamol	no CQ 126.5 mg metronidazole	no CQ 14.1 mg metronidazole	no CQ 1.6 mg paracetamol 14.6 mg metronidazole

FIGURE 1. Falsified samples of chloroquine (CQ) tablets identified in Cameroon and the Democratic Republic Congo, and their thin layer chromatographic (TLC) analysis[11]; see Supplemental Information for details of the analytical procedure. Each TLC plate shows two spots of the respective sample in the middle and two spots of authentic CQ (corresponding to 100% and 80% of the declared amount of the sample) on the left and the right, respectively. Thin layer chromatography plates were photographed in Cameroon and the Democratic Republic Congo with locally available equipment; therefore, the angle of photography is not uniform. The active principles listed at the bottom were identified by high-performance liquid chromatography according to the U.S. Pharmacopeiea and by liquid chromatography–high-resolution mass spectrometry analysis (see text). The CQ amount in sample I was calculated as CQ phosphate; the identity of the counterion (phosphate or sulfate) was not determined. (Photos: packaging, © G. G., C. H., and L. H.; TLC analysis, © F. N. and G. M.)

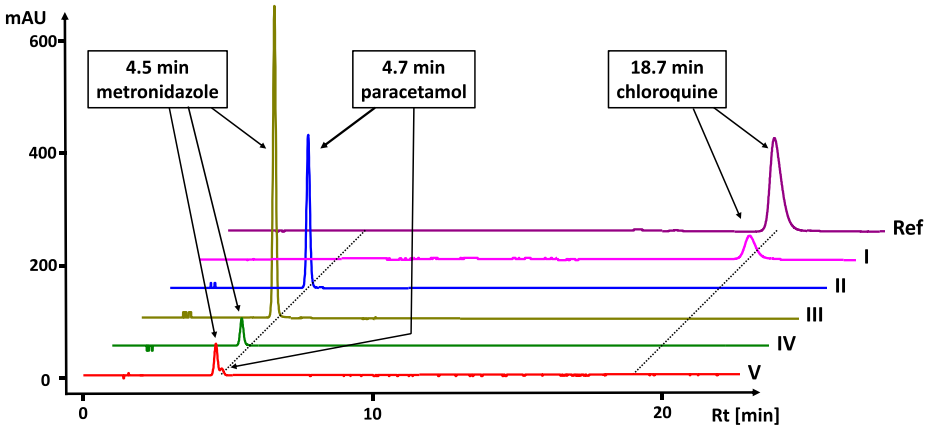

FIGURE 2. High-performance liquid chromatography analysis of falsified samples of chloroquine (CQ) tablets. Analysis was carried out according to the U.S. Pharmacopeia[13]; see Supplementary Information for details of the analytical procedure. Ref = CQ authentic reference substance; I, II, III, IV, and V = falsified samples of CQ tablets (see Figure 1, Table 1).

TABLE 1
Falsified samples of chloroquine tablets identified in Cameroon and the DR Congo

Sample code (Figures 1 and 2)	I	II	III	IV	V
Stated product name	Chloroquine phosphate tablets U.S.P	Chloroquine phosphate tablets U.S.P.	Cloroquine [*sic*] 250 mg	Chloroquine phosphate tablets U.S.P.	Chloroquine phosphate tablets U.S.P
Stated strength	100 mg	100 mg	250 mg	100 mg	250 mg
Stated manufacturer	Jiangsu Pharmaceutical Inc., China	Jiangsu Pharmaceutical Inc., China	Dawa Limited, Kenya	Jiangsu Pharmaceutical Inc., China	Jlangsu [*sic*] Pharmaceutical Inc., China
Batch number, mfg date, exp date	660, August 2018, August 2022	660, May 2017, May 2021	1605059, May 2019, April 2023	660, May 2019, April 2023	660, September 2018, September 2022
Found in	Limbe, Cameroon	Douala, Cameroon	Bukavu, DR Congo	Douala, Cameroon	Douala, Cameroon
Type of facility found in	Private pharmacy	Private pharmacy	Informal vendor	Private pharmacy	Informal vendor
Date of discovery	April 3, 2020	March 31, 2020	April 4, 2020	April 4, 2020	March 31, 2020
Labeling inconsistencies					
Spelling errors	+	–	+	–	+
Invalid NAFDAC registration number	+	+	–	+	+
Same batch number for different products	+	+	–	+	+

DR = Democratic Republic; NAFDAC = National Agency for Food and Drug Administration and Control, Nigeria. NAFDAC registration numbers were checked using the NAFDAC Registered Products Database available at www.nafdac.gov.ng/our-services/registered-products/.

that this sample had not been produced by them. Samples I, II, IV, and V were stated to be produced by "Jiangsu Pharmaceutical Inc., China," but no company with that name, or with the address stated on the labels, could be identified on the internet.

Notably, while this report was in preparation, Cameroon customs authorities reported the seizure of 210 cartons of falsified CQ tablets.[14]

The low amount of CQ in sample I is likely to reflect the attempt by the criminal producers to save costs in the purchase of the active pharmaceutical ingredient. The inclusion of paracetamol, as in sample II, has been reported previously in a falsified medicine from Cameroon, also identified by members of EPN.[10,15] Both in sample II and in that previous case, the amount of paracetamol was too low to achieve a relevant therapeutic effect. Metronidazole is very bitter and was included in samples III, IV, and V probably to mimic the bitter taste of CQ. The antibacterial and antiprotozoal compound metronidazole is usually formulated in tablets of 200–500 mg each. Therefore, samples III, IV, and V contain a subtherapeutic dose, which may contribute to the emergence of antimicrobial resistance. The additional presence of traces of paracetamol in sample V may represent a contamination from a prior production batch, reflecting poor manufacturing standards.

The absence of CQ in four of the five investigated samples, the subtherapeutic amount of CQ in the fifth sample, and the presence of undeclared active pharmaceutical ingredients in four of these samples represent serious health risks for the patients in Cameroon and the DR Congo. The authorities in Cameroon and the DR Congo, and the WHO Rapid Alert System were informed about these findings.

Such products may furthermore cause financial hardships to the patients: sample III was sold in the DR Congo for US$200 for a package of 1,000 tablets, that is, 15 times more expensive than the international procurement price.[16] In Cameroon, the EPN partner organization even reported the occurrence of a further package of 100 CQ tablets with a stated price of 250,000 CFA, that is, US$413 (Supplemental Figure S1, Supplemental Information).

The occurrence of such falsified CQ samples at this time of the COVID-19 pandemic also has wider implications. For any medicine or vaccine which may be reported to be effective against this disease, a frantic demand is to be expected, resulting in a serious danger of the appearance of falsified medicines. Low- and middle-income countries (LMICs) will be especially vulnerable: with their constrained access to essential medicines, their often weak technical capacity for medicine quality assurance and control, and their challenges in the maintenance of appropriate standards of governance in healthcare facilities and national medicines regulatory authorities, they show exactly those conditions which the WHO has identified as favoring the occurrence of substandard and falsified medicines.[17] Because of the recent disruption of the production and supply chains in India and China, which are the most important producer countries of generic medicines for LMICs, this problem will not remain restricted to medicines for the treatment and prevention of COVID-19 but encompass many types of medicines.

The rapid installation of simple, inexpensive screening technologies which can detect substandard and falsified medicines, such as TLC or near infrared or Raman spectroscopy,[8,9,18] may represent an important part of the response to the COVID-19 pandemic in LMICs. The data displayed in Figure 1 are a good example for the possibilities and limitations of the GPHF Minilab[11] in the identification of falsified medicines in future screening programs.

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Supplemental Information for

The identification of falsified chloroquine tablets in Africa at the time of the COVID-19 pandemic

Figure S1: *Package of 100 chloroquine phosphate tablets 100 mg found in Yaoundé, Cameroon, on April 9, 2020, with a stated price of 250,000 CFA, i.e. 414 US $ (Photo: © F. Nyaah). Due to the exorbitant price, this sample was not purchased and not analyzed.*

Methods of thin-layer chromatography and quantitative HPLC analysis

Method	Chloroquine phosphate and sulfate (GPHF Minilab Manual 2020)[1]
Stationary phase	Merck TLC aluminium plates pre-coated with silica gel 60 F_{254}, 5x10 cm
Mobile phase	Ethyl acetate/methanol/25% aqueous ammonia solution 1:4:0.1 (v/v)
Applied volume of sample and standard	2 µl
Detection	1) UV light, 254 nm; 2) Exposure to iodine vapor and visual evaluation in daylight
Standards	Chloroquine phosphate in water, 2.5 and 2.0 mg/ml
Sample preparation	Tablets with a declared content of 100 mg chloroquine phosphate: one tablet was finely ground with a pestle and suspended in 20 ml of water. (Tablets with a declared content 250 mg chloroquine phosphate: one tablet was finely ground with a pestle and suspended in 50 ml of water.) After three minutes of shaking, the solution was allowed to sit for additional five minutes. 2 ml of the supernatant were removed and diluted with 2 ml of water.

Table S1: *Method for thin-layer chromatography*

Method	Chloroquine Phosphate Tablets (USP 42 monograph, 2019)[2]
Instrument	HPLC (Agilent 1100 Series)
Column/stationary phase	Reprospher 100 C18, 250 x 4 mm, 5µm (Dr. Maisch GmbH, Ammerbuch, Germany)
Mobile phase	Methanol/aqueous buffer 22:78 (v/v) (aqueous buffer contained 6.8 g monobasic potassium phosphate and 1 ml perchloric acid per liter water; pH 2.5)
Flow rate	1.2 ml/min
Oven temperature	30 °C
Injection volume	10 µl
Detector	UV, 224 nm
Standard	0.15 mg/ml chloroquine phosphate Pharmaceutical Secondary Standard (Sigma-Aldrich LOT #LRAB3715) in water.
Sample preparation	One tablet was finely ground in a mortar. An aliquot of approx. 100 mg was weighed into a 100 ml volumetric flask. 50 ml of water were added. The flask was sonicated for 15 minutes and then filled up with water to 100 ml. For each sample, two independent experiments were carried out.

Table S2: *Method for quantitative HPLC analysis of chloroquine and paracetamol*

Method	Metronidazole Tablets (USP 42 monograph 2019)[3]
Instrument	HPLC (Agilent 1100 Series)
Column/stationary phase	Reprospher 100 C8, 150 x 4.6 mm, 5µm (Dr. Maisch GmbH, Ammerbuch, Germany)
Mobile phase	Methanol/water 20:80 (v/v)
Flow rate	1.0 ml/min
Oven temperature	30 °C
Injection volume	5 µl
Detector	UV, 254 nm
Standard	0.56 mg/ml Metronidazole Analytical Standard (Sigma-Aldrich LOT #MKBZ3056V) in methanol/water 20:80 (v/v).
Sample preparation	Three tablets were finely ground in a mortar. An aliquot of approx. 100 mg was weighed into a 100 ml volumetric flask. 50 ml of methanol were added. The flask was sonicated for 10 minutes and then filled up to 100 ml with mobile phase. For each sample, two aliquots were weighted and analyzed.

Table S3: *Method for quantitative HPLC analysis of metronidazole*

High resolution liquid chromatography-mass spectrometry

HR-HPLC/MS(/MS) was carried out using a Thermofisher UltiMate 3000 HPLC with a Phenomenex Luna 3µm Polar C18 100 Å column 150 x 2 mm, column temperature 30°C. Eluent A: 0.1% formic acid in water; eluent B: 0.1% formic acid in methanol. Gradient 5-100% B over 20 min followed by 100% B isocratic for 10 min; flow rate 0.3 ml/min. UV detection with a diode array detector. HR mass spectrometry: ESI-TOF Bruker MaXis 4G. The sample solutions were investigated in comparison to authentic paracetamol and metronidazole reference in H_2O/methanol 2:1. In samples III, IV and V, peaks at 5.0 min (metronidazole) were detected. In samples II and V, peaks at 5.4 min (paracetamol) were detected. UV spectra of the samples and the respective references were identical. The molecular ion of the respective samples showed the same exact mass as the molecular ion from paracetamol and/or metronidazole (Table S4). These were consistent with the molecular formula $C_8H_9NO_2$ of paracetamol and $C_6H_9N_3O_3$ of metronidazole.

Sample	Retention time	$[M+H]^+_{theoretical}$	$[M+H]^+_{measured}$	relative mass accuracy
Metronidazole reference	5.0 min	172.0717	172.0719	1.3 ppm
Paracetamol reference	5.4 min	152.0706	152.0709	2.0 ppm
Sample II	5.4 min	152.0706	152.0709	1.8 ppm
Sample III	5.0 min	172.0717	172.0721	2.3 ppm
Sample IV	5.0 min	172.0717	172.0720	1.9 ppm
Sample V	5.0 min	172.0717	172.0718	1.0 ppm
	5.4 min	152.0706	152.0708	1.2 ppm

Table S4: *Retention times, and theoretical and measured exact masses, for the investigated samples and for metronidazole and paracetamol reference substances. HPLC conditions for HPLC-MS are different from those for quantitative analysis according to USP, therefore retention times are different from those shown in Figure 2.*

MS/MS analysis showed the presence of the characteristic fragments of metronidazole (Fig. S2) in samples III, IV and V, and the presence of the characteristic fragments of paracetamol (Fig. S3) in samples II and V. The observed fragmentation of the samples and the respective references were identical.

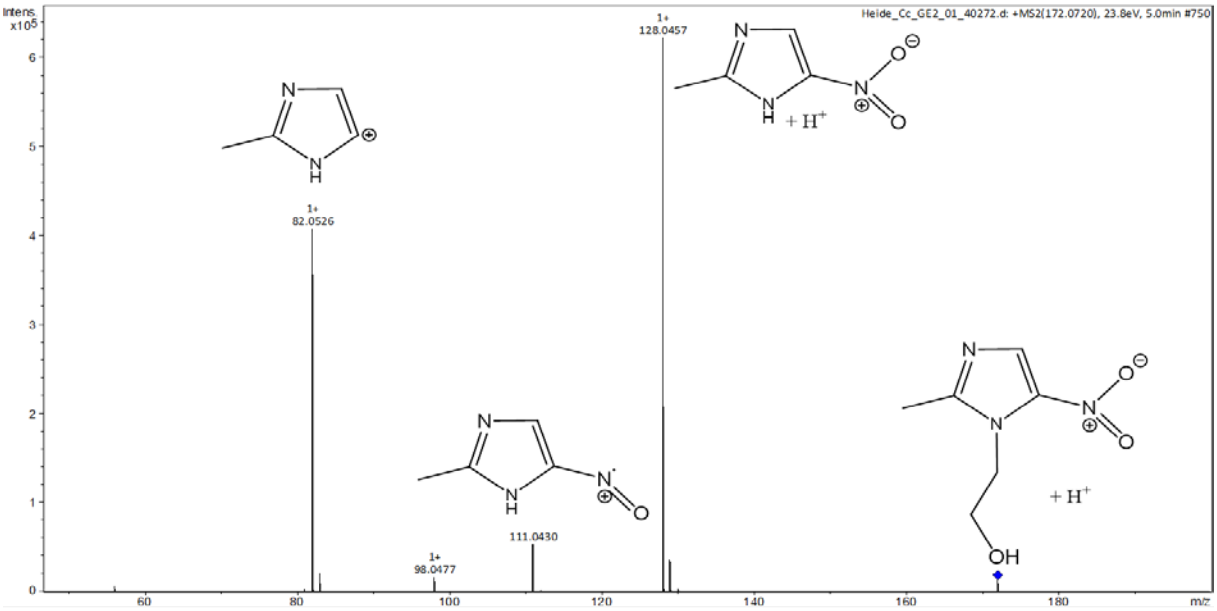

Figure S2: *MS/MS fragmentation of metronidazole in sample IV.*

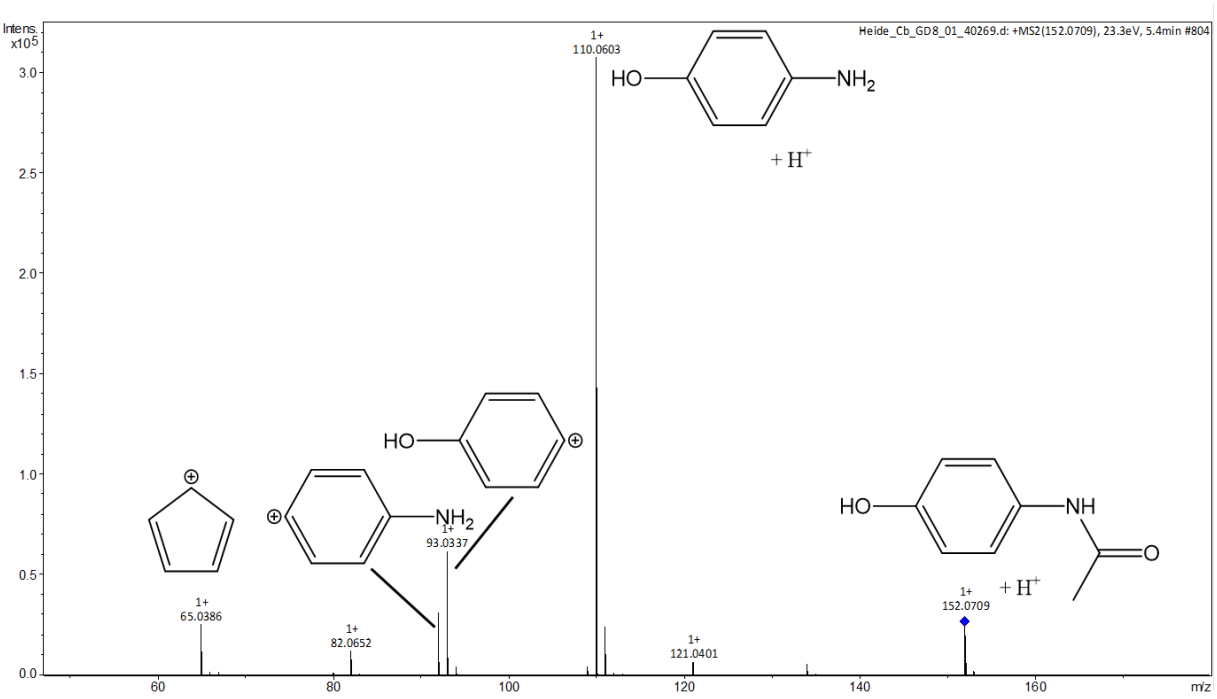

Figure S3: *MS/MS fragmentation of paracetamol in sample II.*

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