Next generation of LFD testing – Smartphone-based Mycotoxin analysis with RIDA®SMART APP

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Abstract

The RIDA®SMART APP is a smartphone application, which was developed as a quantitative and fast on-site/on-field tool for mycotoxin analyses. Typical operational locations are grain mills, feed mills, ethanol plants, flour producers, but also mobile laboratory services that analyze the grain during harvest in the field. Due to the smartphone-based application there are several advantages. It is a small and light device with its own power supply, very versatile, flexible and easy to use. The lab technicians do not need a scientific background, as almost everyone is used to working with a smartphone. Due to the possibility to use mobile networks or WIFI, there are no difficulties in the organization of the collected results. These can either be stored on the smartphone’s internal memory or sent to printers, computers or other devices. Currently, a total of nine different android-based smartphones from various manufacturers are compatible with the RIDA®SMART APP. In combination with one of the compatible smartphones, the RIDA®SMART APP is able to generate results with a CV ≤ 13%, even at highly varying lighting conditions. The recovery of certified reference corn samples, previously analyzed with HPLC or LC-MS/MS, shows a mean value between 91% - 119%. In conclusion it is shown, that the RIDA®SMART APP is highly suitable for an inexpensive, quick and accurate quantitative measurement of mycotoxins in agricultural products (e.g. aflatoxins in corn).

Introduction

The RIDA®SMART APP is a new technology for the quantitative evaluation of lateral flow test (LFD), which are used for immunochromatographic analysis of mycotoxins.

It was developed as an inexpensive, fast and uncomplicated software application for mycotoxin analysis in agricultural products which demands the use of a smartphone and basic laboratory equipment. It was a major requirement that the handling of the RIDA®SMART APP and the LFD tests itself should be as intuitive and simple as possible, but still of the highest possible precision, accuracy and safety. Our experience is, that most customers are under great time pressure (e.g. incoming goods inspection in mills or grain silos) and they need a mobile test system with which they can use quickly and reliably. Many LFD Readers are benchtop devices and need an allocated space. Commonly LFD readers can only store the generated data internally or have to be connected to the local network and are therefore locally bound and not very mobile. This point was also taken into account during the development of the RIDA®SMART APP, as LFD test systems are often used for on-site testing where the only possibility for a network connection is wireless. The software application in combination with a compatible smartphone, analyses the LFD strip by a smartphone-taken picture and quantifies the mycotoxin.
contamination within 4 seconds using a lot specific calibration curve which can be found on the certificate of analysis and can be uploaded to the RIDA®SMART APP. The results are automatically stored in the internal database or can be sent immediately to a printer, computer or other device. In conjunction with a compatible smartphone and RIDA®QUICK Mycotoxin RQS lateral flow tests, the RIDA®SMART APP can measure and quantify results below or at legal EU threshold levels. The software application has been extensively validated by analyzing LFD tests using naturally contaminated reference material. In consequence, the data were directly comparable to an HPLC reference method. In addition, visits to customers and on site testings continuously improve the quality and handling of the RIDA®SMART APP.

To make working with the APP as efficient and easy as possible, each RIDA®QUICK Mycotoxin RQS test kit comes with a specific certificate of analysis and an LFD cover. On the certificate of analysis as well as on the cover, a QR-Code allows the user to enter all important data such as the standard curve and lot specific data, making manual input obsolete and less susceptible to human-borne typing errors. Customer information and sample IDs can be easily scanned and transferred with the Smartphone and integrated barcode scanner. All it takes is to scan the barcodes and the APP links the information to the specific field. To our best knowledge, the RIDA®SMART APP is the only smartphone-based method for quantitative mycotoxin analysis worldwide combined with a broader measuring range as most usual lateral flow analyzers.

To illustrate how the RIDA®SMART APP works, the following describes the testing and handling of the software application using validation data from various smartphones and the R-Biopharm AG test kit R5209 RIDA®QUICK Aflatoxin RQS ECO. It is the aim of the paper to demonstrate the following performance characteristics: the use of different smartphones under different lightning conditions and the comparison to a reference method by using certified reference materials.

**Material & Methods**

**Material:**

(a) **Smartphones and Android versions.**—Smartphones listed in the following are compatible with the RIDA®SMART APP. The operating system must be at least Android version 7.1.

- Google smartphones: Nexus 6, Nexus 6P, Pixel, Pixel 2, Pixel XL
- Motorola smartphones: Moto X PRO, Lenovo Moto G6, Lenovo Moto 1S
- Xiaomi smartphones: Xiaomi RedMi Note 7

(b) **Test kits.**—R5209 RIDA®QUICK Aflatoxin RQS ECO test kit comes with 20 test strips (Figure 1), 45 ml extract solution (10-fold concentrate), 20 ml dilution buffer (ready to use), a ready to use lot-specific evaluation strip cover for RIDA®SMART APP and a lot-specific certificate of analysis that contains a QR-code for the standard curve and other lot-specific data.
1. (c) **Equipment.**—Grinder, scale, graduated cylinder, 1 l bottle, 100 μl - 1000 μl pipette and latest version of the RIDA®SMART APP software (Art. No. ZRSAM1000). As well as a Shaker (e.g. Rock-it 360, Trilogy® Analytical Laboratory Inc. or equivalent), a Centrifuge (≥ 2000 g) and a RIDA®SMART APP 3D Stand (smartphone holder) are required.

2. (d) **Further reagents.**—Distilled or deionized water.

3. (e) **Certified Reference Materials.**—Naturally contaminated certified reference materials from Trilogy® Analytical Labs. The total aflatoxin contaminations (sum of aflatoxin B1, B2, G1, G2) of these materials was measured by the manufacturer by repeated HPLC or LC-MS/MS testing. The experiments described below were carried out with corn samples only.

**Method:**

To obtain the ready-to-use extraction buffer, transfer 1 part of the extraction solution (10-fold concentrate) into 9 parts of distilled or deionized water and homogenize for 5 min. Before extraction, grind and homogenize a representative sample (in accordance with official sampling regulations). Weigh in 10 g of the homogenized sample into a re-sealable tube and add 20 mL of ready-to-use extraction buffer. Shake vigorously for 5 min on a shaker, centrifuge 3 min at 2000 g to obtain a particle-free supernatant. For a measurement range of 2 – 75 μg/kg, 100 μL of the particle-free supernatant can be used in the test. To obtain an extended measurement range of 50 – 300 μg/kg, 100 μL of the particle-free supernatant must be diluted with 700 μL dilution buffer and mixed carefully. Use 100 μL of this dilution in the test.

Take a RIDA®QUICK Aflatoxin RQS ECO test strip and apply 100 μl sample to the application area (sample) of the test strip (Figure 1). Incubate the test strip for exactly 5 minutes at room temperature (20–25°C/68–77°F). For evaluation, open the RIDA®SMART APP on a compatible smartphone [See Material section (a)] place the strip cover on the LFD test strip (Figure 2).

Choose the “Measurement” button, define the sample, the customer ID and scan the QR-code on the strip cover to upload the test name and lot information. Next, scan the QR-code on the certificate of analysis to upload the lot-specific standard curve. By pressing the button “next” you will be forwarded to the image acquisition. Place the picture of the test strip with the strip-cover on it within the predefined markings on the smartphone screen and press the camera button. After autofocusing, the picture is taken and evaluated within seconds (Figure 3).

The measurement is automatically recorded and displayed with all important information, like date and time, sample ID, customer ID, product name, lot number and contamination in ppb (or ppm). Once the results are obtained, it is possible to send them immediately to a printer, computer or other devices.
The left-hand line in the reaction area is the test line. Its intensity depends on the aflatoxin concentration of the sample. The right-hand line in the reaction area is the control line and must appear after each test procedure (See Figure 1).

**Result and Discussion**

To characterize the comparability of five different smartphones (Google Nexus 6P, Goolge Pixel 2, Google Pixel XL, Motorola Lenovo Moto G6, Xiaomi RedMi Note 7), three dried test strips with moderate to strong test lines were measured at highly varying lighting conditions (with five replicates). The strips were measured in four different conditions (hand held with bright artificial light (around 2200 lux), hand held with moderate artificial light (around 1900 lux), hand held with bright natural light (around 1100 lux) and on a RIDA®SMART STAND under laboratory conditions (around 1800 lux). The smartphone was reoriented after each measurement to readjust the focus of the camera. It was also held freehand over the strips to take the photo to be analyzed, as well as placed on a RIDA®SMART STAND so that the camera could always capture the test strip at exactly the same distance (Figure 4).

Table 1 shows the CV of the test line for each of the three tested strips at all four different lighting conditions in percent [%]. The majority of the individual test strip CVs for each light condition is ≤6%. If the results of all five smartphones are combined, the test line CV for test strip 1 is 4%, for test strip 2 it is 6% and test strip 3 it is 6%. It can also be seen that the RIDA®SMART APP is capable of analyzing each of the three test strips at the given lighting conditions with an overall test line CV between 5–13% (Table 1). The overall test line CV shows all measurements of a test strip for each smartphone at all four lighting conditions occurring in the experiment.

Using the RIDA®QUICK Aflatoxin RQS ECO test in combination with the RIDA®SMART APP it is possible to detect aflatoxin contamination between ≥2–300 µg/kg.

A comparison of the test performance with HPLC was carried out by testing Trilogy® naturally contaminated reference material corn samples. The total aflatoxin contaminations (sum of aflatoxin B1, B2, G1, G2) of these materials were measured by the manufacturer by repeated HPLC or LC-MS/MS testing.

Eight Trilogy® corn samples were extracted three times each and tested with two production lots of RIDA®QUICK Aflatoxin RQS ECO according to the instruction for use of the test kit. The results were evaluated with the RIDA®SMART APP installed on a compatible smartphone [See Material section (a)]. The assigned total aflatoxin values of the Trilogy® corn samples given on the certificates were set as target values. The recovery rates for the RIDA®QUICK Aflatoxin RQS ECO evaluated with the RIDA®SMART APP are shown in Table 2. As can be seen, the recoveries vary from 83% to 132% between the individual (single) measurements. The mean value of the recoveries is between 91–119%.
In conclusion it was shown, that the RIDA®SMART APP in combination with RIDA®QUICK Aflatoxin RQS ECO is highly suitable for an inexpensive, quick and accurate quantitative measurement of aflatoxins in corn. It is also proven, that it provides reliable and precise results at any time. Even with highly varying artificial lighting conditions.
Tables and figures:

Figure 0: Cover picture if possible/required

Figure 1: R5209 RIDA®QUICK Aflatoxin RQS ECO test strip.

Figure 2: RIDA®SMART APP strip cover is placed on the LFD test strip.

Figure 3: Taking a picture of a LFD test strip with cover. The picture is processed and analyzed automatically by the RIDA®SMART APP.
Figure 4: LFD strip with cover is being analysed by the RIDA®SMART APP. Therefore, the smartphone is placed in a RIDA®SMART STAND.

Table 1: Five smartphones (Google Nexus 6P, Goolge Pixel 2, Google Pixel XL, Motorola Lenovo Moto G6, Xiaomi RedMi Note 7) were used to characterize for comparability; each of the tested smartphones was used to analyse each of the three dried test strips in five replicates and four different lighting conditions (hand held with moderate artificial light (around 1900 lux) [1], on a RIDA®SMART STAND under laboratory conditions (around 1800 lux) [2], hand held with bright natural light (around 1100 lux) [3] and hand held with bright artificial light (around 2200 lux) [4]). The table shows the test lines CV for each of the three tested strips at all four different lighting conditions in percent [%].

| Lighting condition | Test strip 1 | Test strip 2 | Test strip 3 |
|--------------------|-------------|-------------|-------------|
|                    | Nexus 6P    | Pixel XL    | Pixel 2     | Redmi7      | Moto 6G     |
| 1                   | 6%          | 1%          | 3%          | 1%          | 1%          |
| 2                   | 2%          | 0%          | 2%          | 1%          | 2%          |
| 3                   | 4%          | 2%          | 1%          | 2%          | 1%          |
| 4                   | 9%          | 2%          | 2%          | 9%          | 2%          |
| Overall CV          | 13%         | 9%          | 10%         | 8%          | 10%         |

|                    | Nexus 6P    | Pixel XL    | Pixel 2     | Redmi7      | Moto 6G     |
| 1                   | 5%          | 1%          | 1%          | 1%          | 1%          |
| 2                   | 1%          | 2%          | 1%          | 3%          | 1%          |
| 3                   | 2%          | 3%          | 1%          | 3%          | 2%          |
| 4                   | 2%          | 4%          | 5%          | 3%          | 5%          |
| Overall CV          | 8%          | 11%         | 13%         | 5%          | 13%         |

|                    | Nexus 6P    | Pixel XL    | Pixel 2     | Redmi7      | Moto 6G     |
| 1                   | 1%          | 0%          | 6%          | 2%          | 5%          |
| 2                   | 2%          | 1%          | 2%          | 4%          | 2%          |
| 3                   | 3%          | 2%          | 6%          | 4%          | 3%          |
| 4                   | N/A         | 2%          | 11%         | 8%          | 3%          |
| Overall CV          | 6%          | 9%          | 13%         | 5%          | 12%         |
Table 2: Recovery of total aflatoxin (sum of aflatoxin B1, B2, G1, G2) in µg/kg using two production lots of RIDA®QUICK Aflatoxin RQS ECO. The assigned total aflatoxin values of the Trilogy® corn samples given on the certificates were set as target values (100 %); three extractions per reference material were undertaken.

| Certified value µg/kg | Lot 1 |     |     | Lot 2 |     |     |     |     |     |     |     |     |
|-----------------------|-------|-----|-----|-------|-----|-----|-----|-----|-----|-----|-----|-----|
|                       | rep. 1| rep. 2 | rep. 3 | rep. 1 | rep. 2 | rep. 3 | mean | SD  | CV (%) |
| 4,2                   | 93%   | 90%  | 92%  | 87%   | 83%  | 100% | 91%  | 6%  | 6,4  |
| 9,3                   | 93%   | 99%  | 102% | 90%   | 99%  | 89%  | 95%  | 5%  | 5,5  |
| 10,6                  | 96%   | 102% | 86%  | 108%  | 105% | 110% | 101% | 9%  | 8,8  |
| 19,2                  | 121%  | 126% | 113% | 131%  | 113% | 108% | 119% | 9%  | 7,4  |
| 87,9                  | 112%  | 110% | 132% | 112%  | 110% | 126% | 117% | 10% | 8,1  |
| 282                   | 94%   | 104% | 132% | 99%   | 97%  | 100% | 104% | 14% | 13,4 |
| 391                   | 94%   | 94%  | 93%  | 93%   | 102% | 101% | 96%  | 4%  | 4,3  |
| 857                   | 105%  | 102% | 96%  | 100%  | 104% | 96%  | 101% | 4%  | 3,9  |
Table 1: Five smartphones (Google Nexus 6P, Google Pixel 2, Google Pixel XL, Motorola Lenovo Moto G6, Xiaomi RedMi Note 7) were used to characterize for comparability; each of the tested smartphones was used to analyse each of the three dried test strips in five replicates and four different lighting conditions (hand held with moderate artificial light (around 1900 lux) [1], on a RIDA®SMART STAND under laboratory conditions (around 1800 lux) [2], hand held with bright natural light (around 1100 lux) [3] and hand held with bright artificial light (around 2200 lux) [4]). The table shows the test lines CV for each of the three tested strips at all four different lighting conditions in percent [%].

| Lighting condition | Test strip 1 | Test strip 2 | Test strip 3 |
|--------------------|--------------|--------------|--------------|
|                    | Nexus 6P | Pixel XL | Pixel 2 | Redmi 7 | Moto 6G | Nexus 6P | Pixel XL | Pixel 2 | Redmi 7 | Moto 6G | Nexus 6P | Pixel XL | Pixel 2 | Redmi 7 | Moto 6G |
| 1                  | 6%       | 1%       | 3%     | 1%     | 1%     | 1%       | 1%       | 1%     | 1%     | 1%     |
| 2                  | 2%       | 0%       | 2%     | 1%     | 2%     | 2%       | 1%       | 1%     | 1%     | 2%     |
| 3                  | 4%       | 2%       | 1%     | 2%     | 1%     | 2%       | 1%       | 1%     | 1%     | 2%     |
| 4                  | 9%       | 2%       | 2%     | 9%     | 2%     | 1%       | 1%       | 1%     | 1%     | 2%     |
| Overall CV         | 13%      | 9%       | 10%    | 8%     | 10%    | 8%       | 11%      | 13%    | 5%     | 13%    |

| Lighting condition | Test strip 2 | Test strip 3 |
|--------------------|--------------|--------------|
|                    | Nexus 6P | Pixel XL | Pixel 2 | Redmi 7 | Moto 6G | Nexus 6P | Pixel XL | Pixel 2 | Redmi 7 | Moto 6G |
| 1                  | 5%       | 1%       | 1%     | 1%     | 1%     | 1%       | 1%       | 1%     | 1%     | 1%     |
| 2                  | 1%       | 2%       | 1%     | 3%     | 1%     | 2%       | 1%       | 1%     | 1%     | 2%     |
| 3                  | 2%       | 3%       | 1%     | 3%     | 2%     | 2%       | 1%       | 1%     | 1%     | 2%     |
| 4                  | 2%       | 4%       | 5%     | 3%     | 5%     | 2%       | 2%       | 1%     | 1%     | 2%     |
| Overall CV         | 8%       | 11%      | 13%    | 5%     | 13%    | 8%       | 9%       | 13%    | 5%     | 12%    |
Table 2: Recovery of total aflatoxin (sum of aflatoxin B1, B2, G1, G2) in µg/kg using two production lots of RIDA®QUICK Aflatoxin RQS ECO. The assigned total aflatoxin values of the Trilogy® corn samples given on the certificates were set as target values (100 %); three extractions per reference material were undertaken.

| Certified value µg/kg | Lot 1       |          | Lot 2       |          |
|-----------------------|-------------|----------|-------------|----------|
|                       | rep. 1 rep. 2 rep. 3 | mean SD CV (%) | rep. 1 rep. 2 rep. 3 | mean SD CV (%) |
| 4,2                   | 93% 90% 92% | 87% 83% 100% | 91% 6% 6,4 |
| 9,3                   | 93% 99% 102% | 90% 99% 89% | 95% 5% 5,5 |
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