EFFECT OF ACIDIC HYDROLYSIS ON SULFAMIDES DETECTION IN HONEY WITH NEW GENERIC ANTIBODY-BASED DIPSTICK ASSAY

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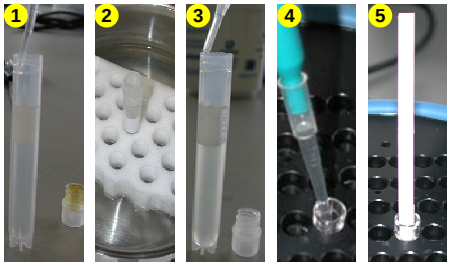
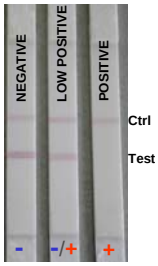
I. ABSTRACT

Sulfamides (or sulfonamides) constitute a large family of synthetic antimicrobial drugs largely used to prevent and treat animal and bee diseases. These drugs may cause a variety of human harmful reactions, including strong allergy reaction, urinary tract and haemopoietic disorders. Due to their extensive use, there is a need to verify the potential presence of their undesired residue in different food products including honey.
Unfortunately, there is a lack of generic rapid tests able to detect all sulfamides compounds at the same time. Moreover, due to chemical binding between honey sugars and sulfamides, most of existing sulfamides screening tests require a long procedure of honey hydrolysis step before analysis.

II. DESCRIPTION OF THE TEST

We have therefore developed a new rapid test based on dipstick format. We have also checked the necessity to apply an acidic hydrolysis step before the sample analysis. This indirect competitive Lateral Flow (LF) assay uses specific generic monoclonal antibodies and the result of the test is directly visualized on specific capture lines by the use of colloidal gold-conjugates. Our results show that the hydrolysis step is mandatory to detect sulfamides in honey with our system. We have thus developed a new short and easy "on field" hydrolysis protocol. Our final test detects more than 10 sulfamides in honey at low concentration level (< 25 ppb for most of sulfamides). It only requires a very easy sample hydrolysis procedure. The adapted hydrolysis protocol takes only a 5 minutes incubation in boiling water and the overall dipstick analysis takes 20 minutes.

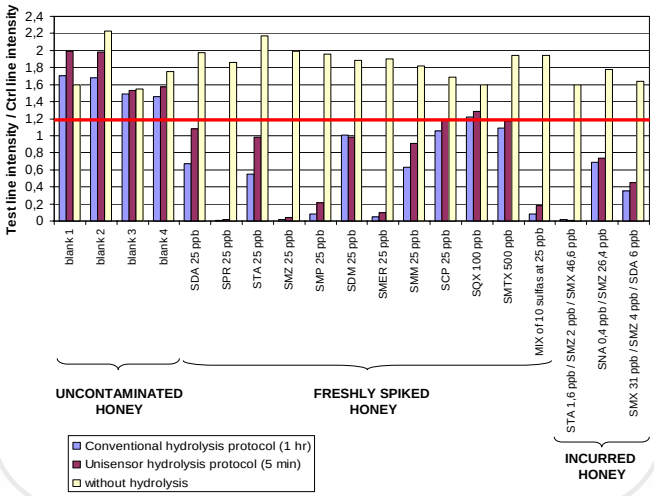




1. ***650 mg*** *Honey + Acid buffer*
2. ***5 min*** *in boiling water*
3. *Neutralizing + Honey buffers*
4. ***200 µl*** *sample + reagent* ***5 min*** *at* ***40°C***
5. *Dipstick for* ***15 min*** *at* ***40°C***

III. RESULTS

Results of dipstick analyses of blank and contaminated honey samples showing the importance of the hydrolysis step prior sulfamides detection. The 5 min-hydrolysis protocol newly developed allows the detection of the main sulfamides at 25 ppb (µg/kg) concentration.



IV. SENSITIVITY

Sulfapyridine :	< 25 ppb
Sulfamethazine :	< 25 ppb
Sulfamethoxypyridazine :	< 25 ppb
Sulfamerazine :	< 25 ppb
Sulfamonomethoxine :	< 25 ppb
Sulfadiazine :	25 ppb
Sulfadimethoxine :	25 ppb
Sulfathiazole :	25 ppb
Sulfachloropyridazine :	25 ppb
Sulfaquinoxaline :	150 ppb
Sulfamethoxazole :	500 ppb
Sulfadoxine :	>> 1000 ppb
Sulfacetamide :	>> 1000 ppb
Sulfanilamide :	No detection

V. OPTIONAL ACCESSORIES

A combined Heatsensor (**a**) allows to perform automatically the analysis in 1 single step. An optical Readsensor (**b**) gives automatically results of the test, suppressing any subjectivity from the user.

VI. CONCLUSION

In conclusion, we have developed a rapid, easy and generic screening test able to detect, in one single analysis, more than 10 sulfamides in honey.

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