Bioprofiling of cosmetics with focus on streamlined coumarin analysis

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Highlights

- *Sensitive and selective new method to determine coumarin even in matrix-rich samples down to 1.3 mg kg^{-1}*
- *Fast and simple sample preparation: Direct solving or suspension of the samples in oxolane with 5 min ultrasonic extraction*
- *Rapid method with an analysis time below 4 min allows to screen a high number of samples on the market*
- *Additional gain in information by profiling of active cosmetic ingredients using various assays (effect-directed analysis, EDA)*
- *Hyphenation of HPTLC with MS enables the assignment of unknown active cosmetic ingredients*

Stable fluorescence of derivatized coumarin

- ✓ Dark storage guaranteed stable fluorescence and good reproducibility (0.7-3.7%).
- ✓ Stabilization with polyethylen glycol substantially increased sensitivity (Fig. 1).
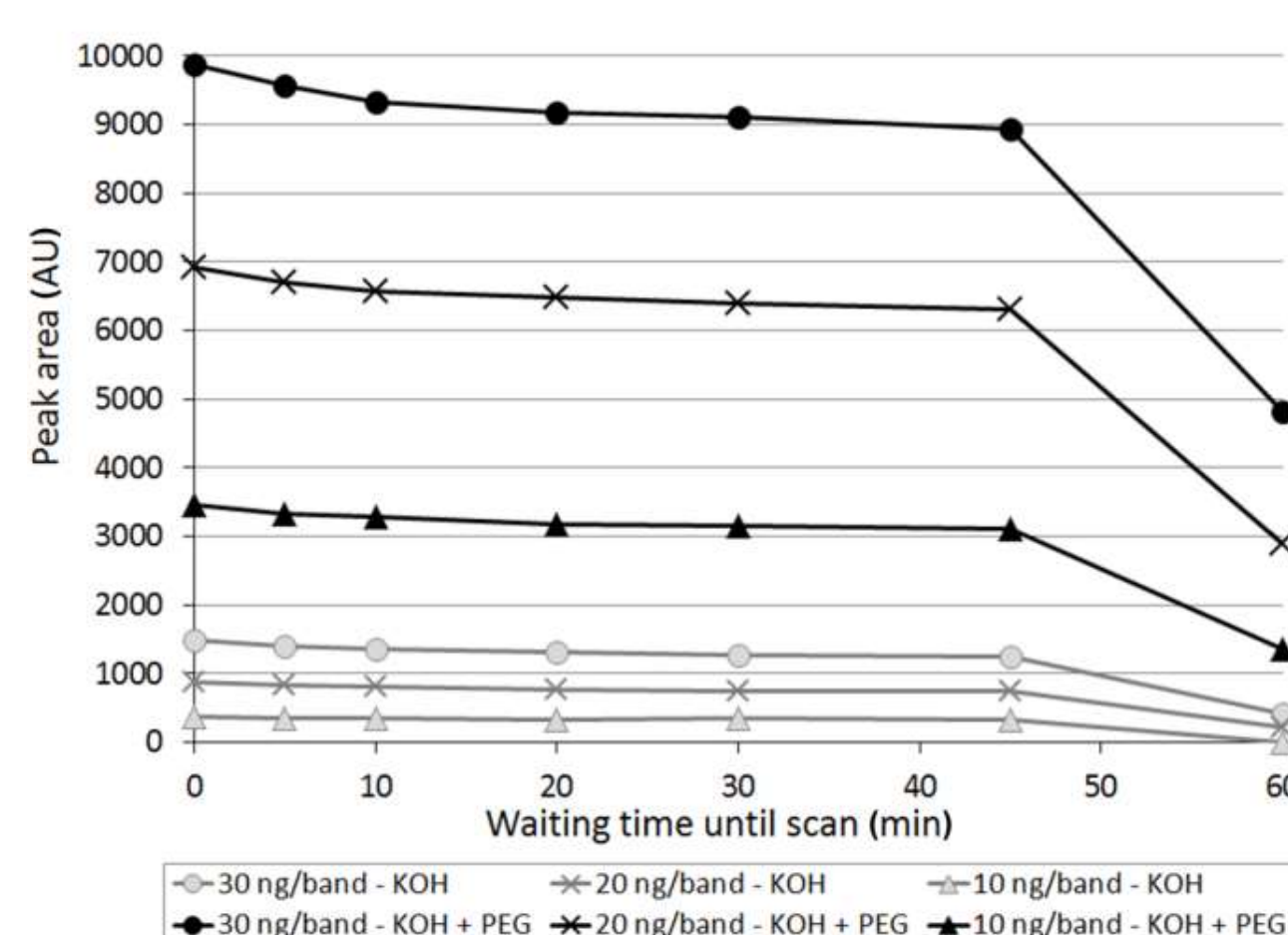

Fig. 1 Fluorescence stability of coumarin: After development, plate was halved and either derivatized with potassium hydroxide (KOH, grey lines) or by additional stabilization with PEG solution (black lines), fluorescence measurement after storage in the dark (0-45 min) and in daylight for 15 min before last measurement (application volumes 10-30 ng band^{-1})

Selective determination of coumarin in cosmetics

- ✓ The mobile phase n-hexane, ethyl acetate and ammonia (25%) 3.8:1.3:0.05 (V/V/V) enabled a selective and even specific detection of coumarin.
- ✓ Despite the minimal sample preparation, a baseline separation from matrix components was evident.
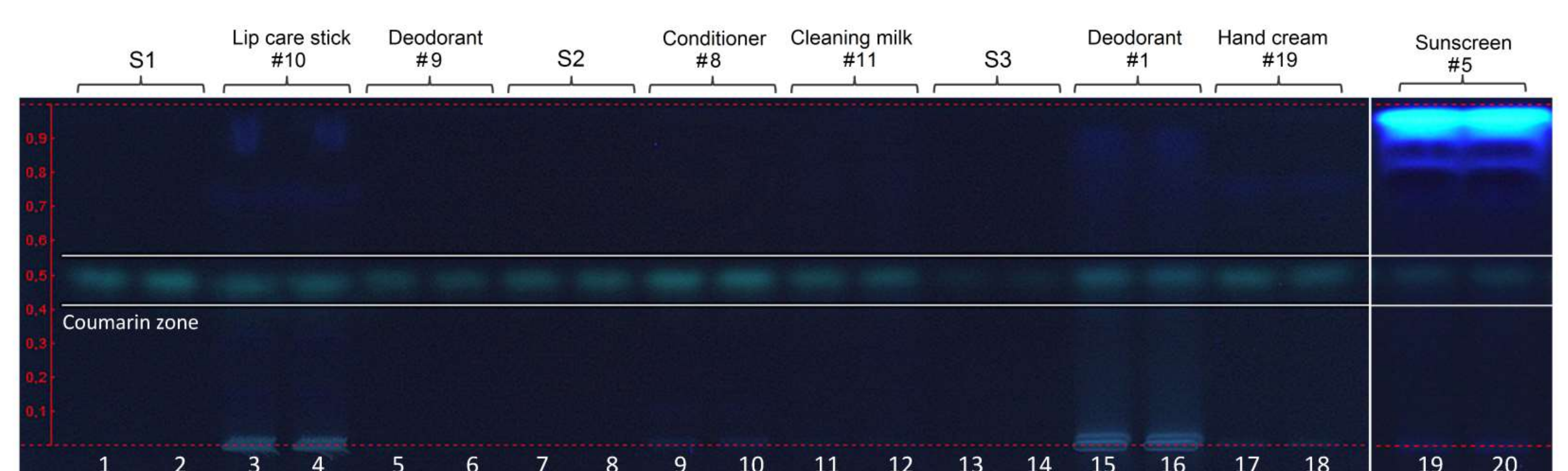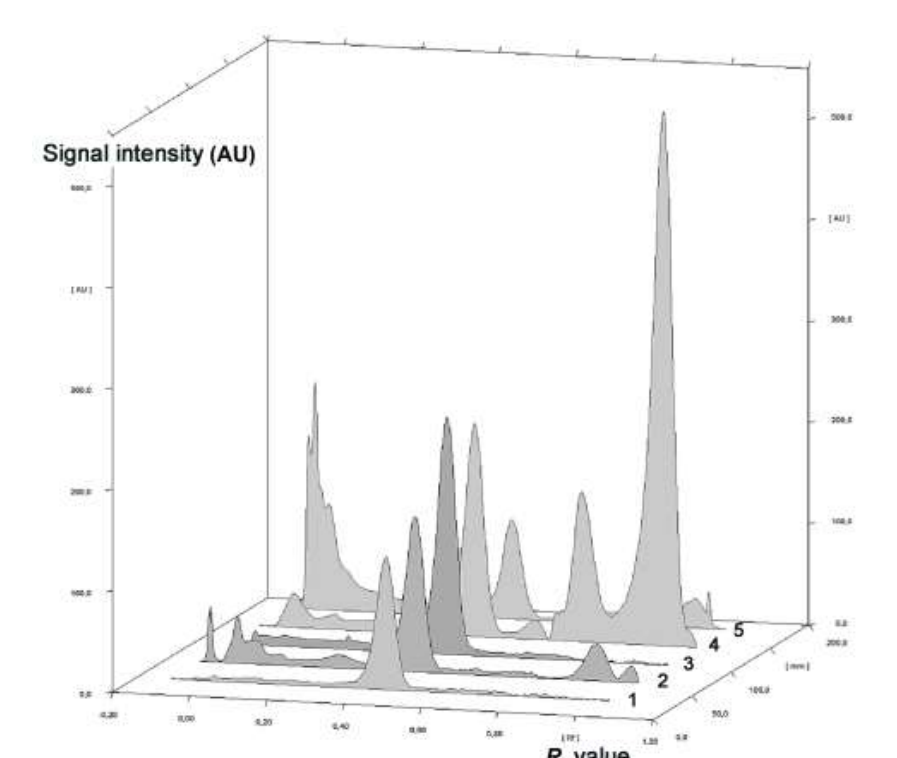- ✓ Analysis of up to 17 cosmetic samples in parallel (Fig. 2)

Fig. 2 HPTLC chromatogram at UV 366 nm of coumarin at hR_F 50 in different cosmetics (twofold overall analysis) with matrix mainly located at the start zone or in the front, as well as coumarin standard (S1-S3, 2.5-20 ng band^{-1}).
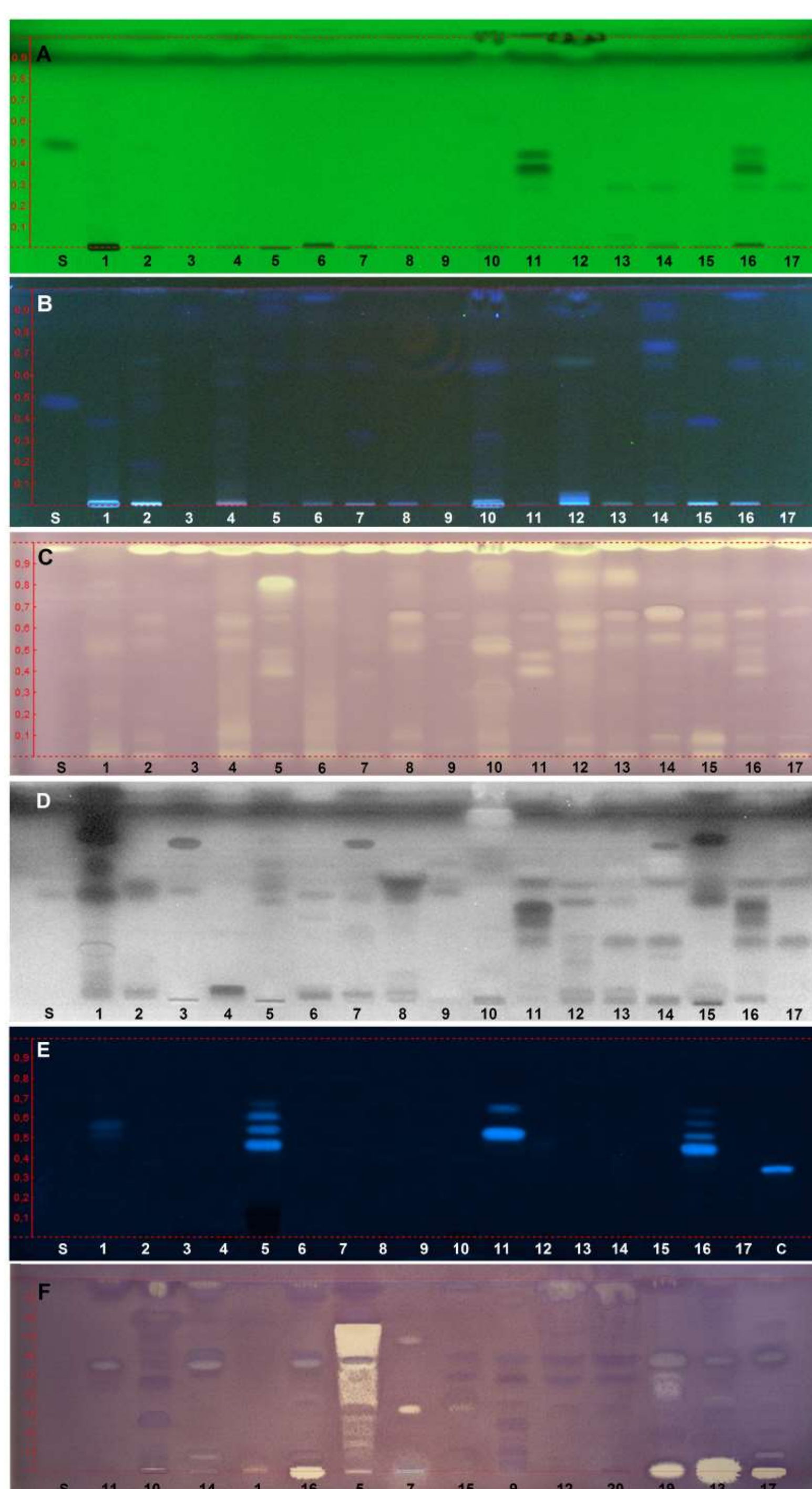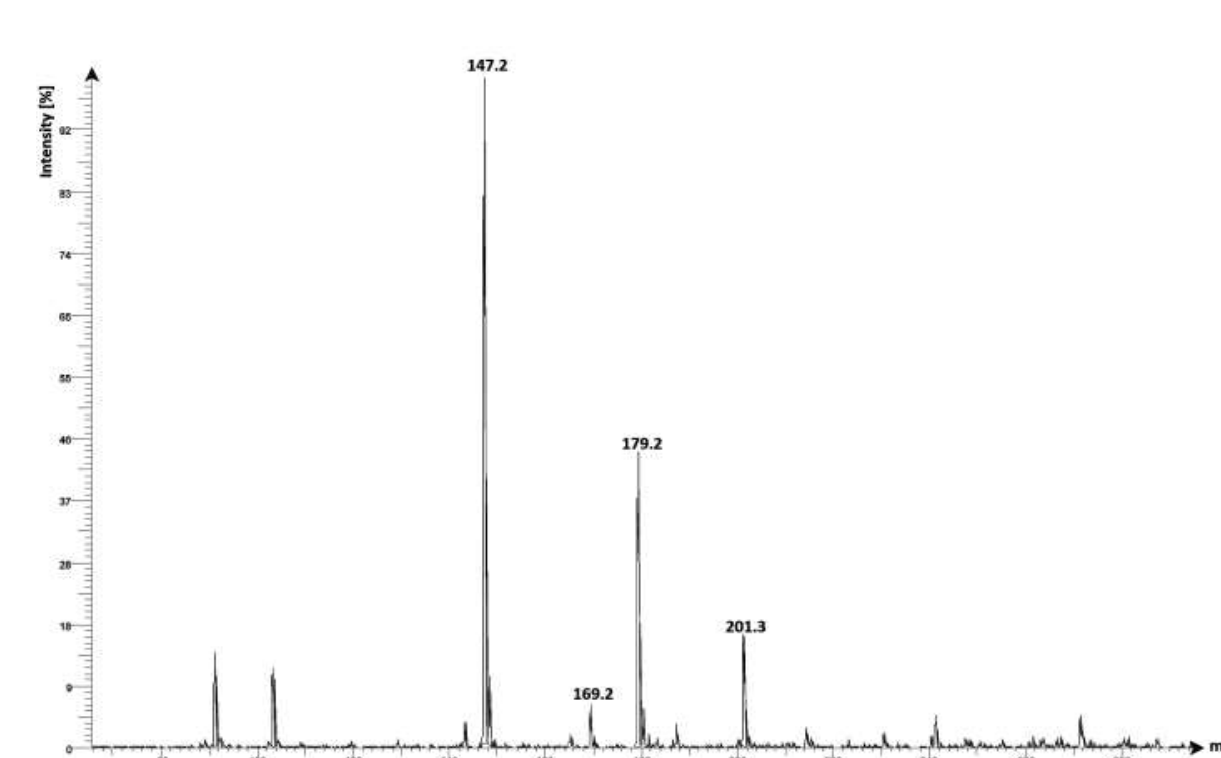

Fig. 4 Mass spectrum of a coumarin zone in a cosmetic sample showing as basepeak the protonated molecule at *m/z* 147 and its sodium adduct at *m/z* 169

Bioprofiling of cosmetics

- ✓ Assays provide an additional gain in information for coumarin and other active substances (Fig. 3).
- ✓ Results can be used for risk assessment of cosmetic ingredients and products.
- ✓ HPTLC-MS supported the assignment of unknown bioactive ingredients (Fig. 4).
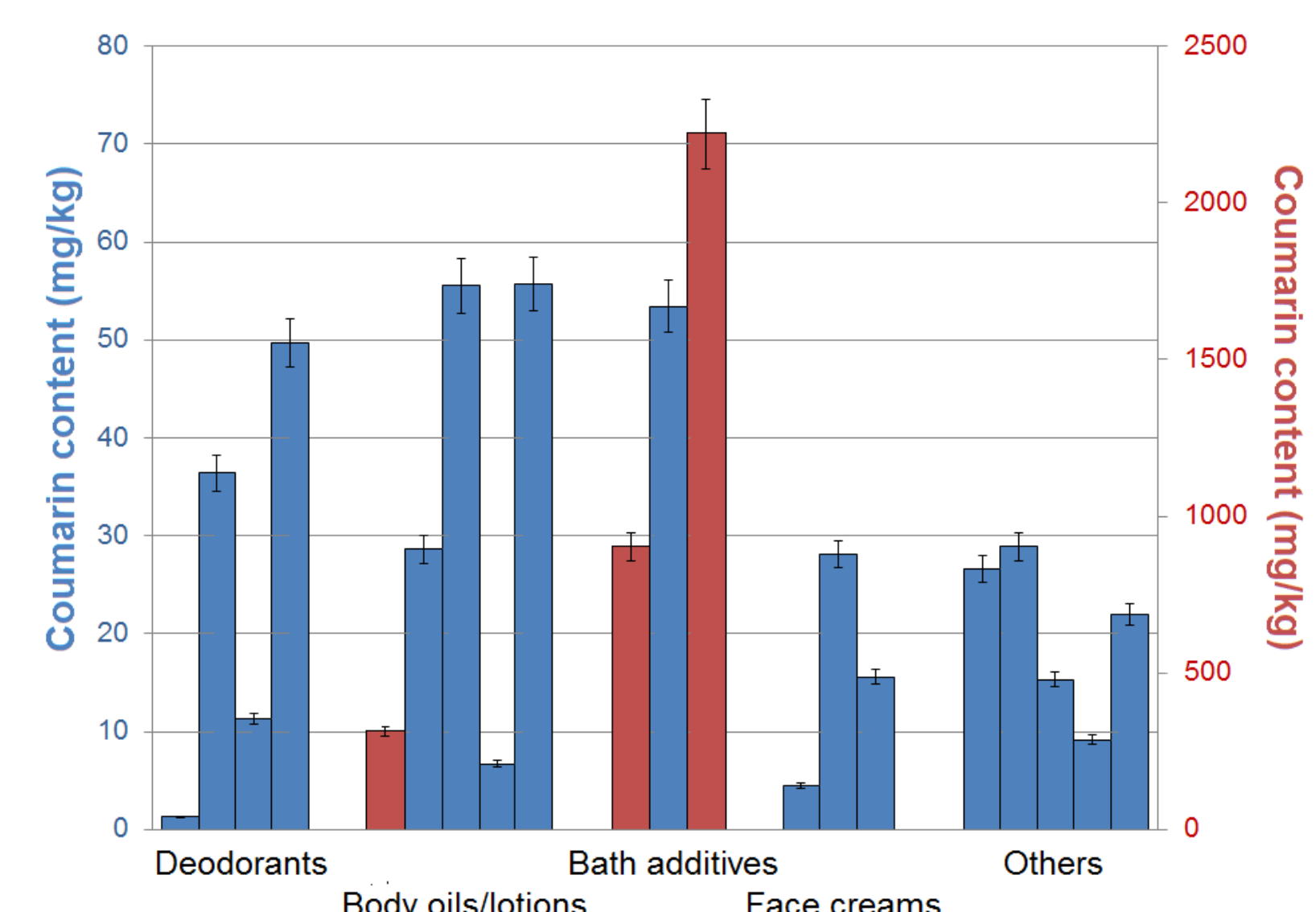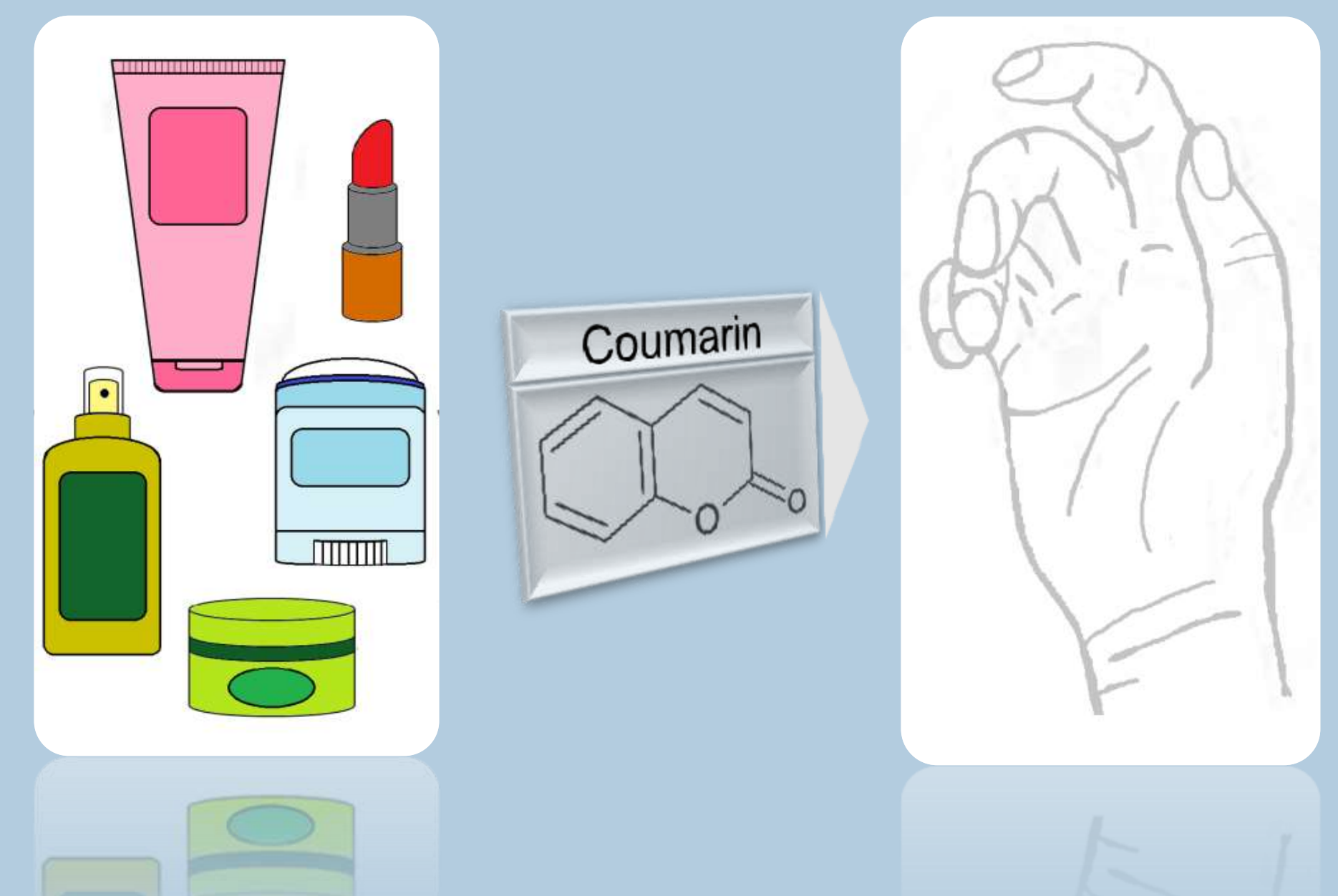- ✓ Confirmation of the endocrine potential of parabens

Fig. 3 HPTLC-EDA chromatograms of 17 cosmetics of different formulations and coumarin standard (S, 800 ng band^{-1}) at UV 254 nm (A), UV 366 nm (B) and after DPPH• assay at white light illumination (C) as well as bioautogram after *A. fischeri* bioassay as greyscale image (D), pYES bioassay at UV 366 nm (E, with estradiol 150 pg band^{-1} as positive control C) and *B. subtilis* bioassay at white light illumination (F)

Fig. 5 Overview of the determined coumarin content of the different cosmetic samples (blue: low contents of coumarin up to 55 mg kg^{-1}; red: high contents up to 2218 mg kg^{-1})

Quantification of coumarin in 20 cosmetic samples

- ✓ Applicability of the method was proven for a wide range of cosmetic formulations (Fig. 5).
- ✓ The coumarin content varied between 1.3 and 2218 mg kg^{-1}.
- ✓ It was shown that cosmetic products can contribute substantially to the overall exposure to coumarin.

Reference [1] Stiefel, C., Schubert, T., Morlock, G.E. (2017) Bioprofiling of cosmetics with focus on streamlined coumarin analysis, in submission.

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