Streamlined structure elucidation of unknowns in formulations
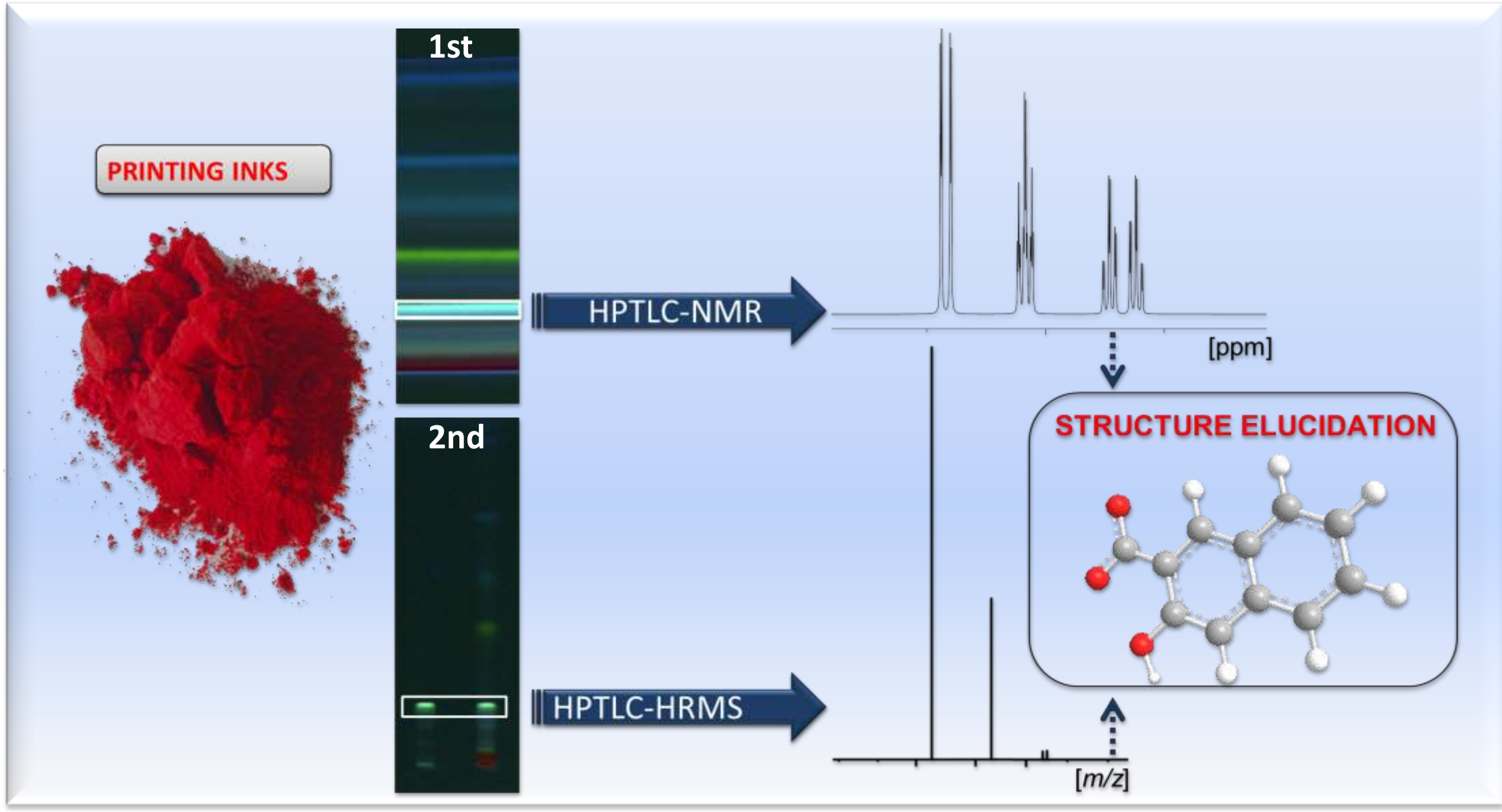
I. Yüce, G.E. Morlock   Justus Liebig University Giessen

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Fig. 1 Analysis of PR 57:1, batch 1472, and elucidation of an unknown compound

Highlights

- *Fast and reliable quality control for pigment formulations and characterization of an unknown compound in pigment Red 57:1 to be 3-hydroxy-2-naphtoic acid*
- *Streamlined workflow for structure elucidation*
- *Determination of an unknown with only one analytical and one preparative AMD run*
- *Hyphenation of powerful tools: HPTLC-UV/vis/FLD-HRMS and PLC-IR/NMR (Fig. 1)*
- *9-times higher target load on plate achieved via a selective extraction (discrimination of matrix) and increased band sharpness via a 9-step AMD 2 gradient*

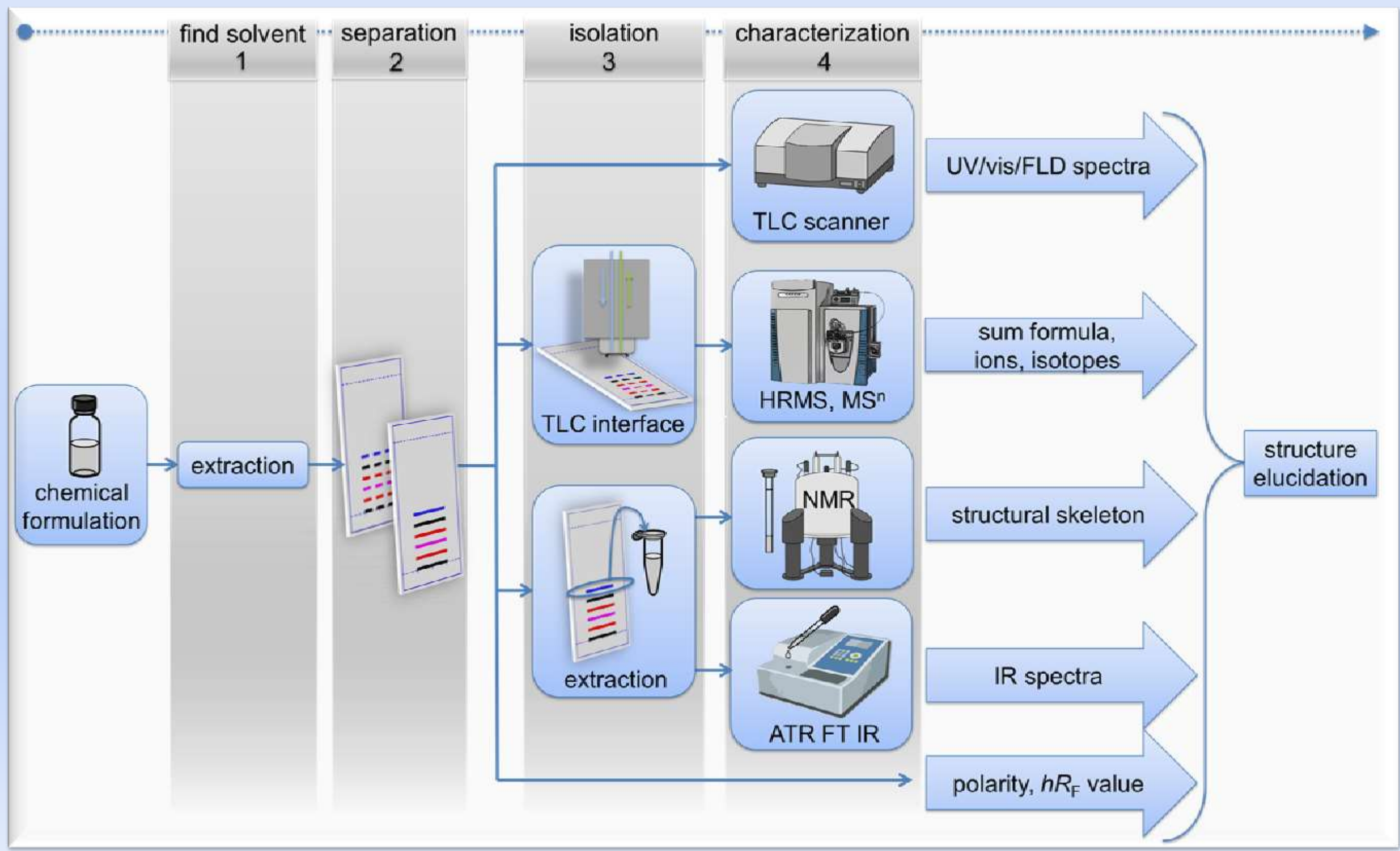## Workflow for streamlined structure elucidation and solvent screening

Fig. 2 Streamlined workflow for structure elucidation by planar chromatography

- Four steps of streamlined structure elucidation, using only one analytical and one preparative layer (Fig. 2)
- Selective solvent screening to maximize the load of the target band on the preparative layer (Fig. 3)
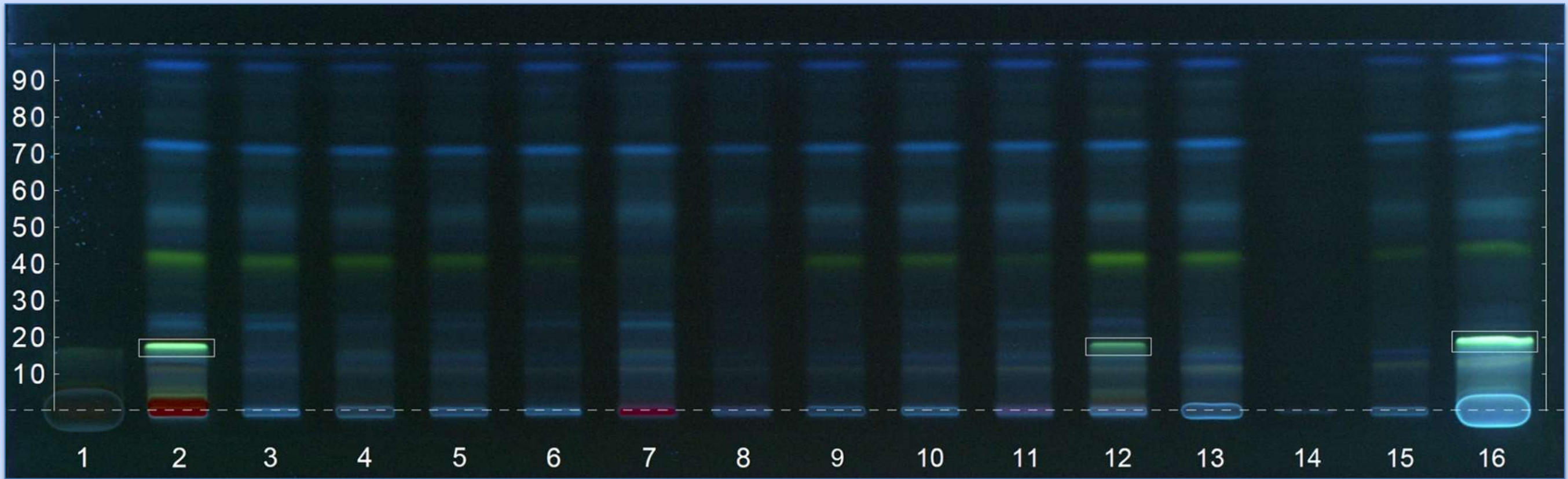

Fig. 3 HPTLC chromatogram at UV 366 nm showing 16 different extracts of pigment formulation PR 57:1, batch 1472 (band of interest is highlighted); extraction with bi-distilled water (1), methanol (2), acetone (3), 1,4-dioxane (4), 2-butanone (5), diethylether (6), chloroform (7), toluene (8), acetonitrile (9), ethyl acetate (10), dichloromethane (11), tetrahydrofuran (12), 2-butanol (13), n-hexane (14), i-propyl acetate (15) and acetic anhydride (16)

Characterization by HPTLC-UV/vis/FLD-HRMS/MS² and PLC-IR/-NMR

HPTLC-UV/vis/FLD

Verification of the unknown compound in different PR 57:1 batches (Fig. 4 a-c, tracks 2–5) via standard substance (track 1, hR_F 17)

PLC-IR/-NMR

Spectra of the unknown compound (Figs. 4 e and 5)

HPTLC-HRMS/MS²

- Unknown compound measured with a mass dependent error of 0.1 ppm (Fig. 4 f-i)
- MS² confirmed the CO_2 loss
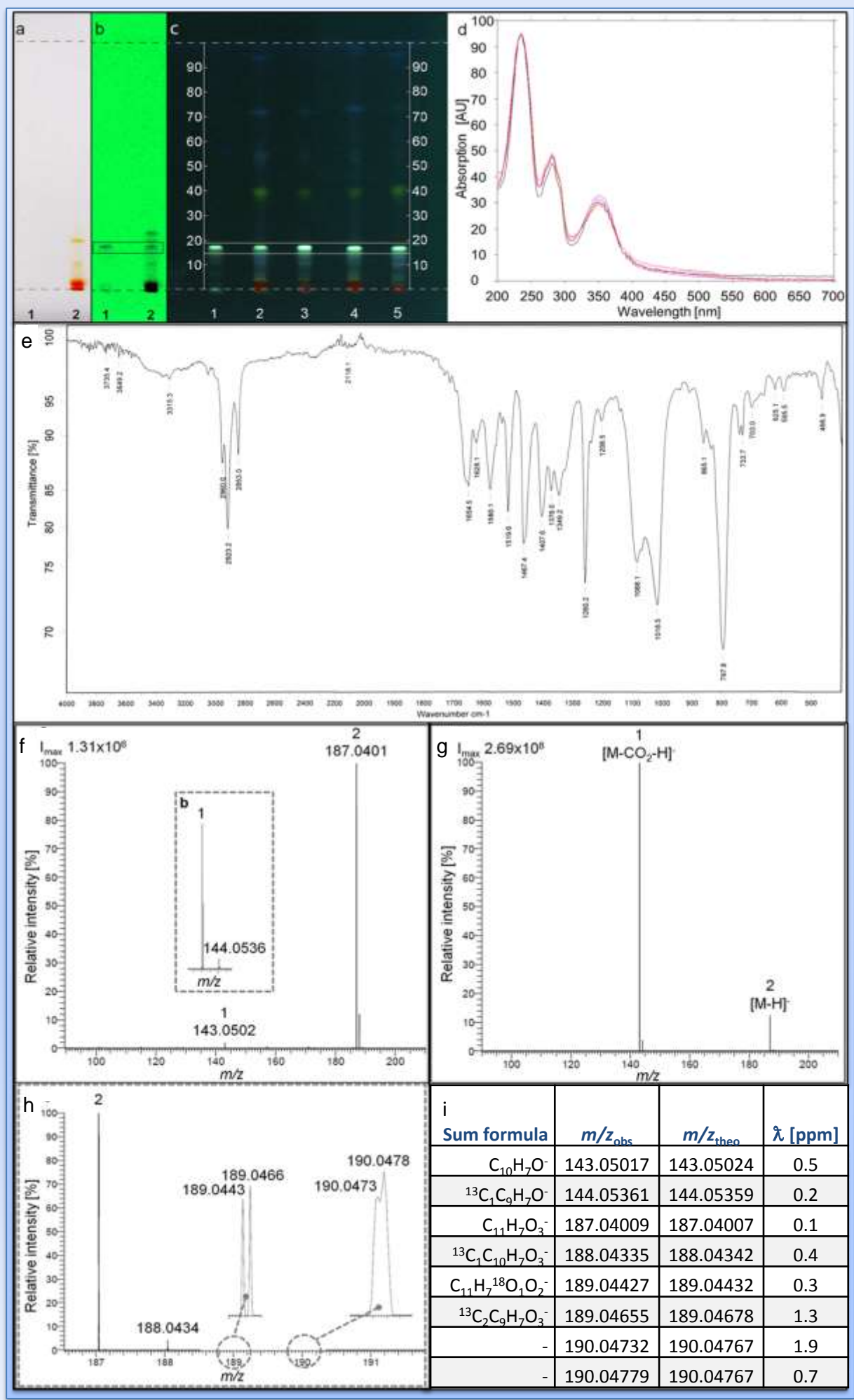

Fig. 4 HPTLC-vis/UV/FLD (a-c), absorption spectra (d), PLC-IR (e) and HPTLC-HRMS/MS² (f-i)

Results

✓ Unknown is 3-hydroxy-2-naphtoic acid!

✓ Only one analytical layers needed for HPTLC-UV/vis/FLD-HRMS/MS²

✓ Only one preparative plate needed for 1- and 2-dimensional NMR

✓ Streamlined workflow for structure elucidation based on planar chromatography

✓ Automated preparative gradient development to reach sharp bands and direct scale up to preparative layer

✓ Yield for NMR analysis improved by a factor of 9 via selective extraction
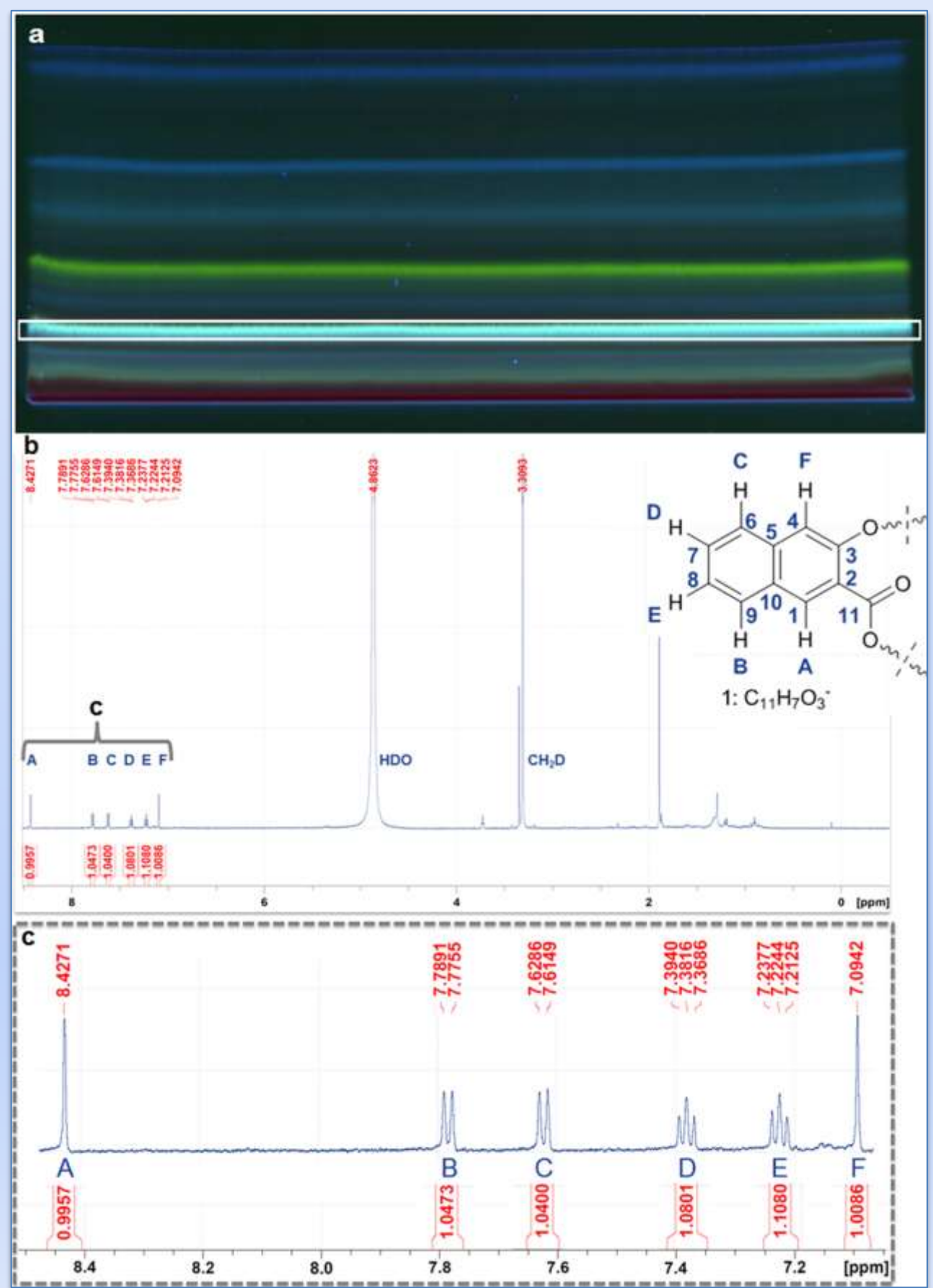

Fig. 5 Preparative chromatogram at UV 366 nm of PR 57:1, batch 1472 (a, marked band of interest); respective PLC-^{1}H NMR spectrum (b) and downfield area with clear signals for the aromatic protons (c, enlarged)

Reference [1] I. Yüce, G.E. Morlock, J Chromatogr A, 1469 (2016) 120-127

IFZ Interdisziplinäres Forschungszentrum Giessen Research Centre for BioSystems, Land Use and Nutrition

Food Science, Justus Liebig University Giessen, Institute of Nutritional Science, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany