

Magnetic solid-phase extraction for the determination of dyes in candies using high performance liquid chromatography coupled to mass spectrometry

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Topic: Synthetic food dyes, widely used for their stability and low cost, have been regulated due to potential health risks, with limits ranging from 50 to 300 mg/kg. Consequently, they are being replaced by natural alternatives like curcumin, which is considered safer and has a limit of 300 mg/kg.

The **objective** of this study was the development of an analytical method for the simultaneous determination of a total of 14 dyes (including both natural and artificial) in candies. For this purpose, magnetic solid-phase extraction (MSPE) was applied for sample treatment and liquid chromatography (LC) coupled to tandem mass spectrometry with triple quadrupole analyzer (MS/MS-QQQ) was applied.

Sample treatment optimization

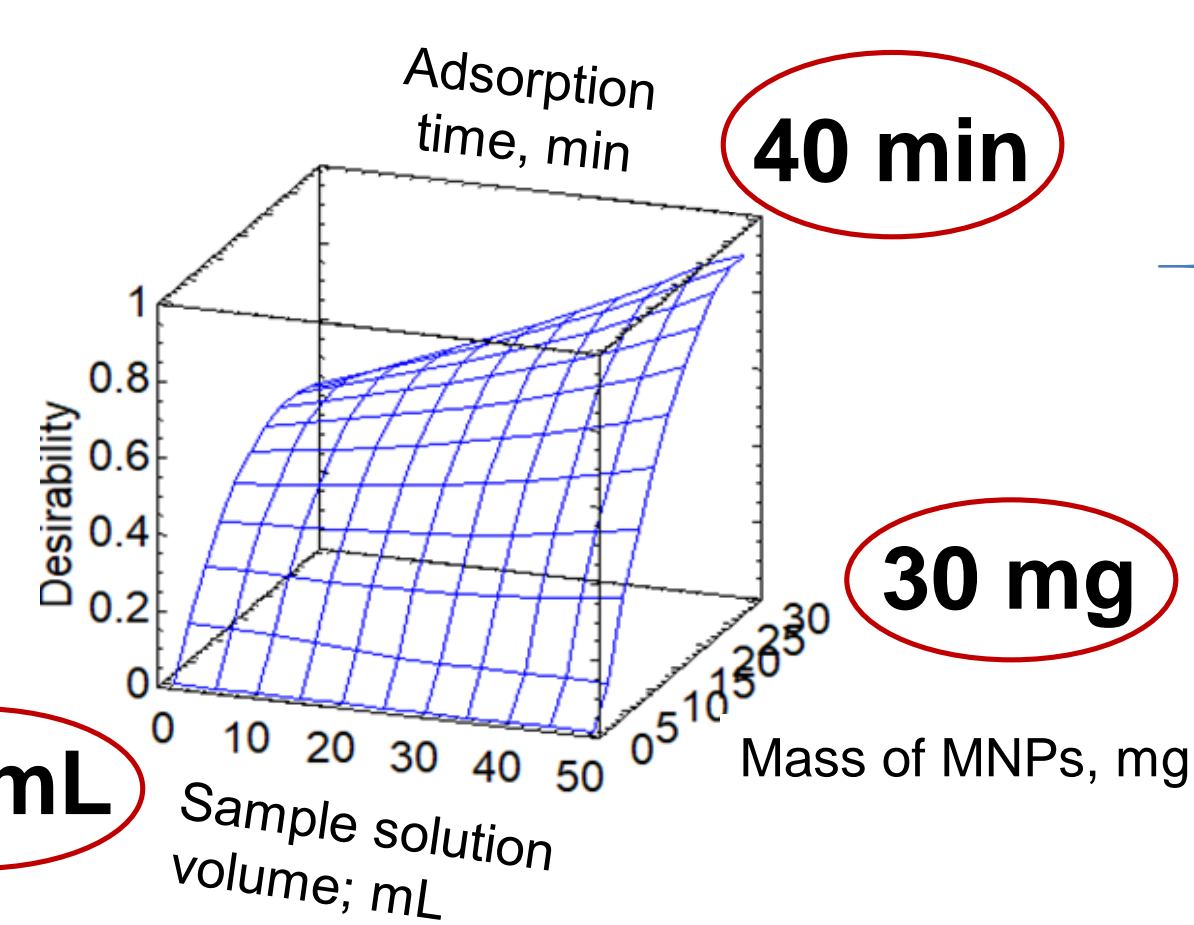
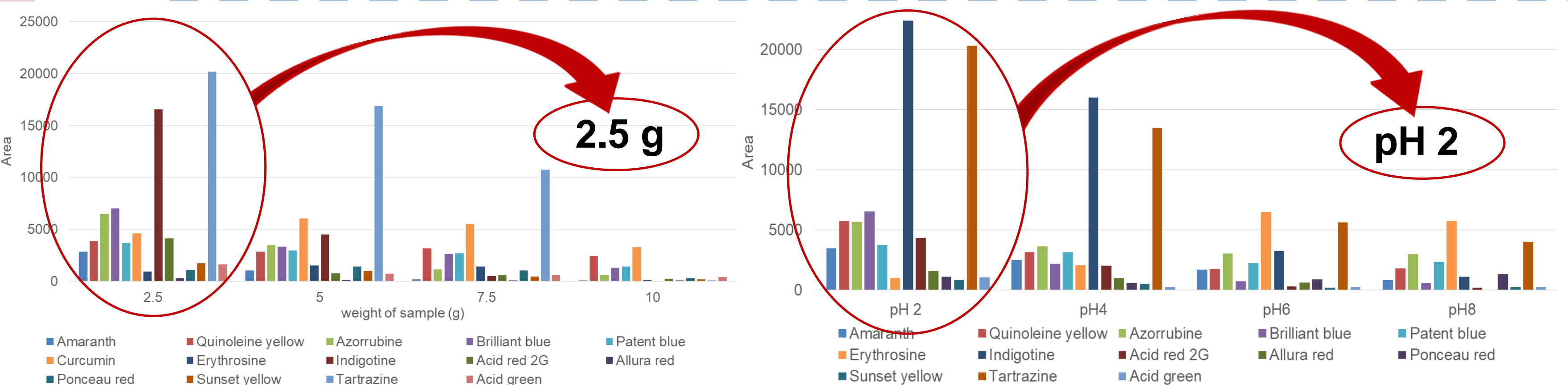
✓ Sample mass: 2.5, 5, 7.5 and 10 g

✓ pH of sample solution: 2, 4, 6 and 8

✓ Types of magnetic nanoparticles (MNPs) assayed:

- Polyaniline@ferrite (PANI@Fe₃O₄)
- Cellulose@Fe₃O₄
- Polyethyleneimine@polydopamine@Fe₃O₄ (PEI@PDA@Fe₃O₄)

Only this material provided preconcentration of the analytes



✓ Mass of PEI@PDA@Fe₃O₄ MNPs: 5, 17.5 and 30 mg

✓ Volume of sample solution: 10, 30 and 50 mL

✓ Adsorption time: 10, 25 and 40 min

✓ Desorption time: 2, 5 and 10 min

✓ Desorption volume: 2, 3 and 4 mL

✓ Desorption solvent:

- Ethanol
- Acetonitrile (ACN)
- 85:15, 70:30, 50:50, 25:75 (v:v) ACN:NH₃ mixtures

❖ ACN:NH₃ (70:30)
❖ 3 mL
❖ 10 min

Proposed sample treatment



2.5 g candy samples

Addition of 100 mL water and heating at 40 °C until dissolution. pH adjustment at 2 with HCl

- (1) 50 mL sample solution
- (2) Addition of 30 mg PEI@PDA@Fe₃O₄

● Dyes
● Matrix sample

(3) Adsorption (40 min)

(4) Separation of MNPs

(6) Desorption (5 min)

(5) 3 mL desorption solvent (70:30 ACN:NH₃)

(7) Filtration and drying

(8) Reconstitution in 0.25 mL of water

Analysis by HPLC-MS/MS

Analytical characteristics of the MSPE with HPLC-MS/MS method

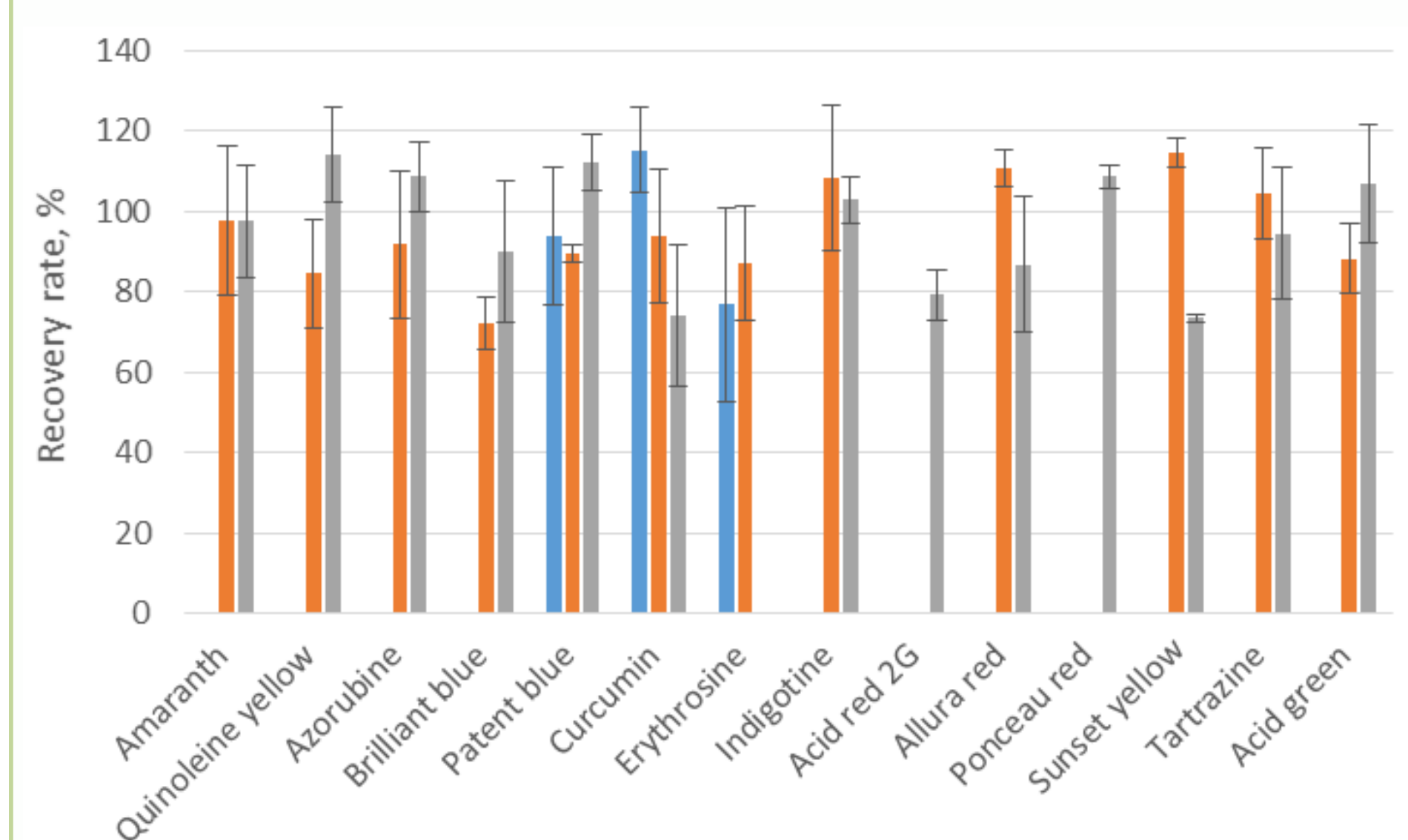
Dye	R ²	LOD (µg/kg)	LOQ (µg/kg)
Amaranth	0.9914	30.4	101
Quinoline yellow	0.9936	1.12	3.72
Azorubine	0.9911	0.52	1.73
Brilliant blue	0.9943	1.56	5.20
Patent blue	0.9918	0.53	1.76
Curcumin	0.9921	0.80	2.67
Erythrosine	0.9927	0.60	2.00
Indigotine	0.9979	47.1	157
Acid red 2G	0.9924	0.98	3.28
Allura red	0.9915	18.5	61.7
Ponceau red	0.996	275	918
Sunset yellow	0.9923	0.38	1.27
Tartrazine	0.9967	37.1	124
Acid green	0.9911	0.03	0.11

LOD: Limit of detection calculated using signal-to-noise ratio (S/N) of 3. LOQ: Limit of quantification calculated using S/N=10.

Repeatability, %RSD (n=3) and recoveries (%) in candies using the proposed method

The results of repeatability at three concentration levels, with RSD lower than 24% in all cases, were between:

○ 77-115% (lower) ○ 72-115% (middle) ○ 74-114% (upper)
0.02-0.2-2 mg/kg 0.05-0.5-5 mg/kg 0.08-0.8-8 mg/kg



Analysis of thirteen different commercial candy samples

- Jelly-beans of different colours, shapes and sizes
- Sugar-coated sweets
- Coloured marshmallows

Contents above the LOQ were obtained for different dyes, between 0.018 - 4.45 mg/kg.

Dye	Concentration ranges in analyzed sweets (mg/kg)
Azorubine	0.37
Brilliant blue	0.018-1.41
Patent blue	0.17
Curcumin	0.039-0.12
Allura red	0.065-1.97
Tartrazine	1.21-4.45

- No signals were detected for amaranth, quinoline yellow, erythrosine, indigotine, acid red 2G, ponceau red, sunset yellow and acid green.
- Signals between the LOD and LOQ were detected in ponceau red for the red marshmallow sample and in tartrazine for two samples (sugar-coated worms and gummy bears).

In **conclusion**, the proposed method based on MSPE with HPLC-MS/MS is suitable for the quantification of 14 dyes in candies. The method is able to quantify concentrations from 0.11 µg/kg to 10 mg/kg, depending on the analyte, with good precision. The optimized extraction allowed accurate results to be obtained using minimal volumes of organic solvents with a low sample mass consumption (2.5 g).