Electronic Supplementary Material (ESI) for RSC Advances.

Paper-based chemical reaction arrays as an effective tool for geographical indication of turmerics

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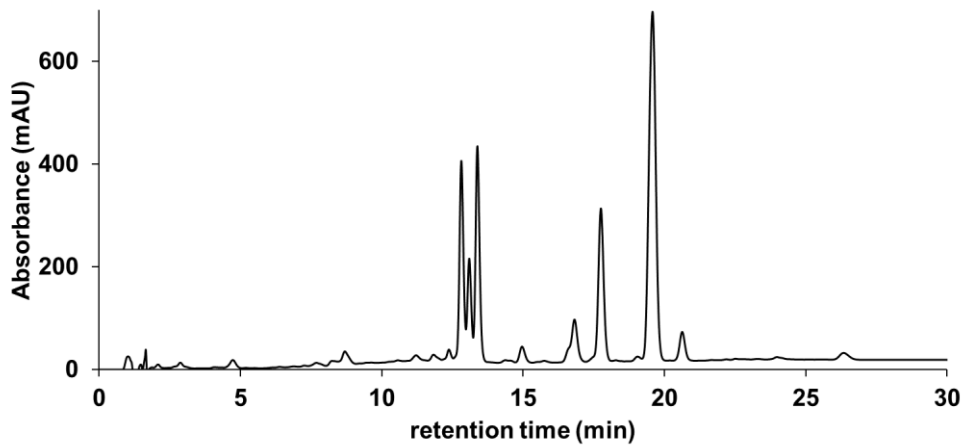
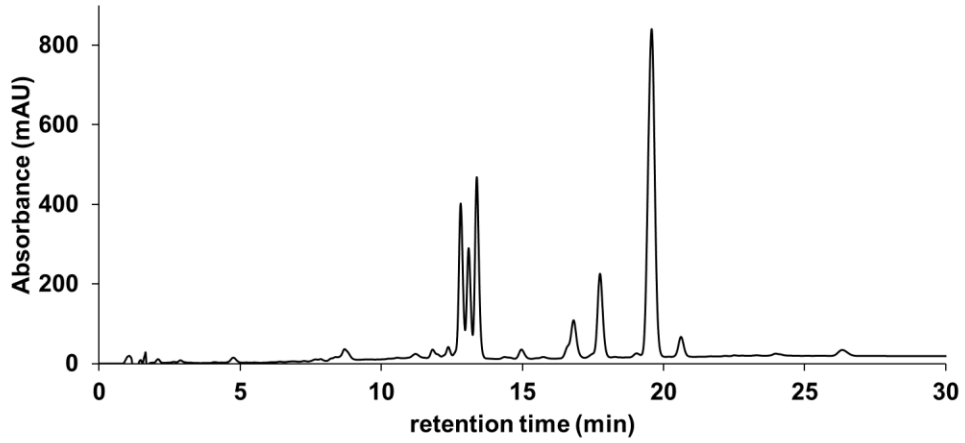
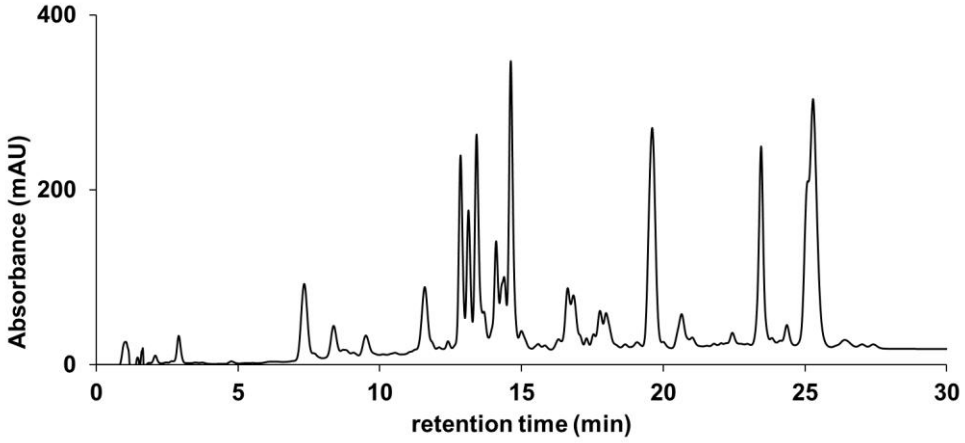
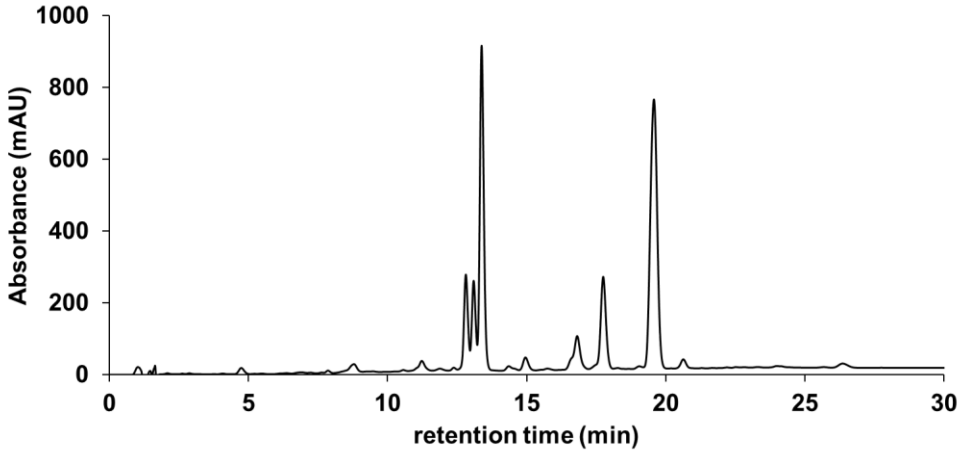
Table S1. The origins of turmeric samples used in this study.

Code	Source Location	Type
Cur1	Chumphon, Thailand	Fresh turmeric
Cur2	Bangkok, Thailand	Fresh turmeric
Cur3	Singapore	Fresh turmeric
Cur4	China	Fresh turmeric
Cur5	Thailand	Turmeric powder
Cur6	Thailand	Turmeric powder
Cur7	Vietnam	Turmeric powder
Cur8	India	Turmeric powder
Cur9	Myanmar	Turmeric powder
Cur10	China	Turmeric powder
Cur11	USA	Standard 98% curcumin

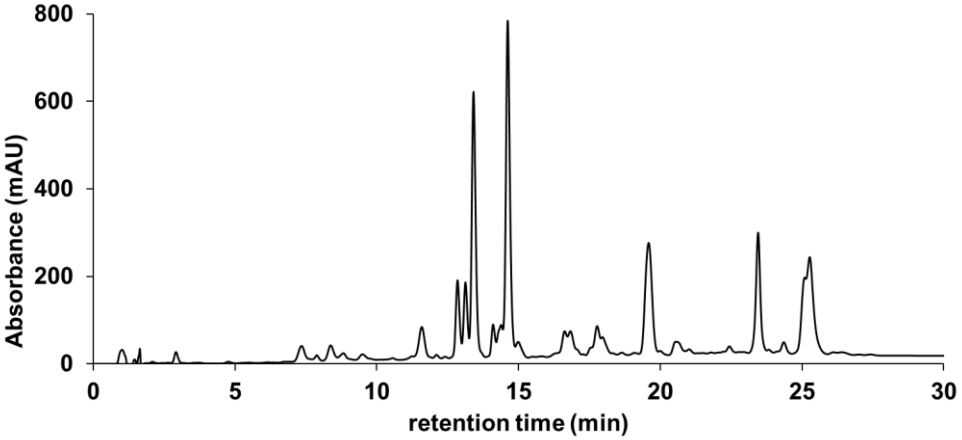
Table S2. Chromatograms of methanol extracts from different sources of turmerics (at 250 nm)

Code	Chromatogram
Cur1	
Cur2	
Cur2 + 2,4-DNP	

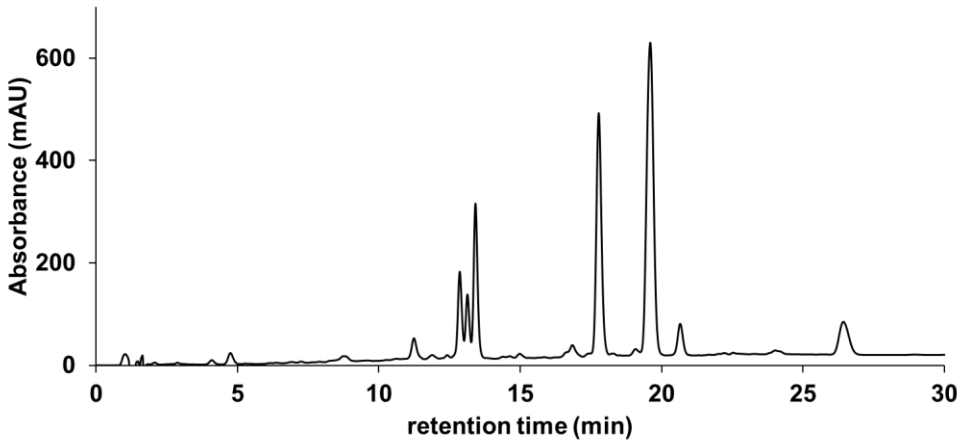
Cur3



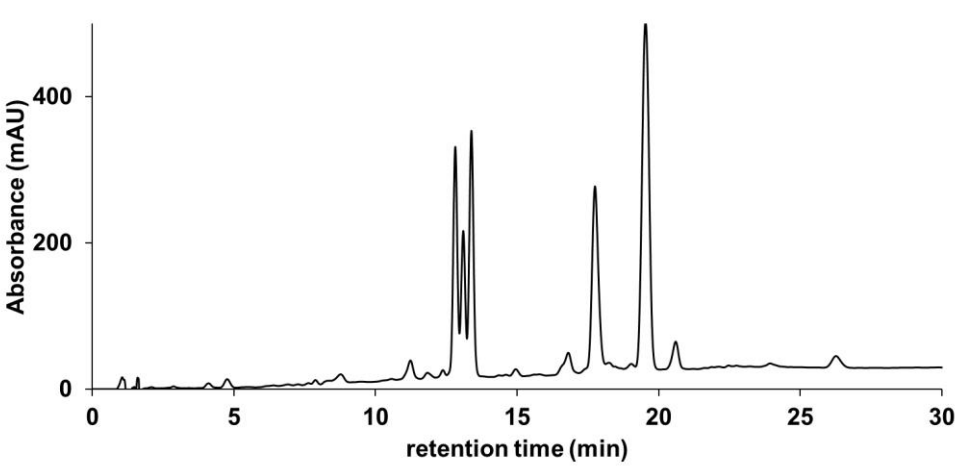
Cur3 + 2,4-DNP



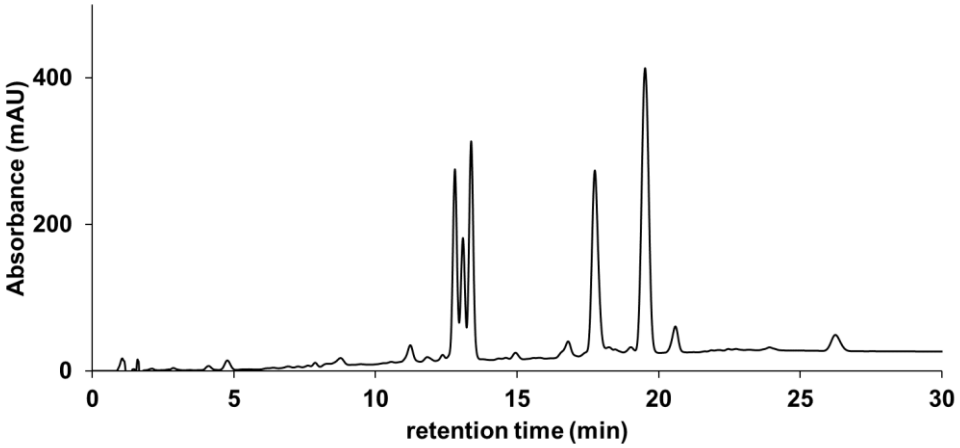
Cur4



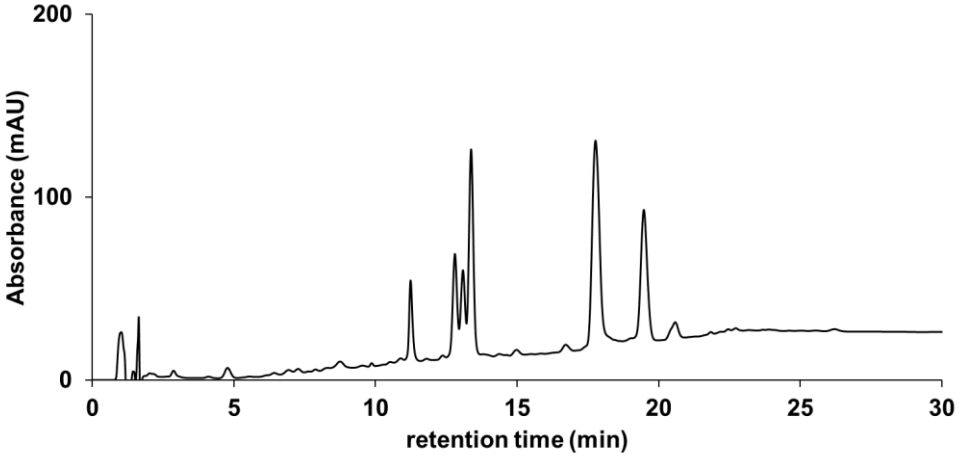
Cur5



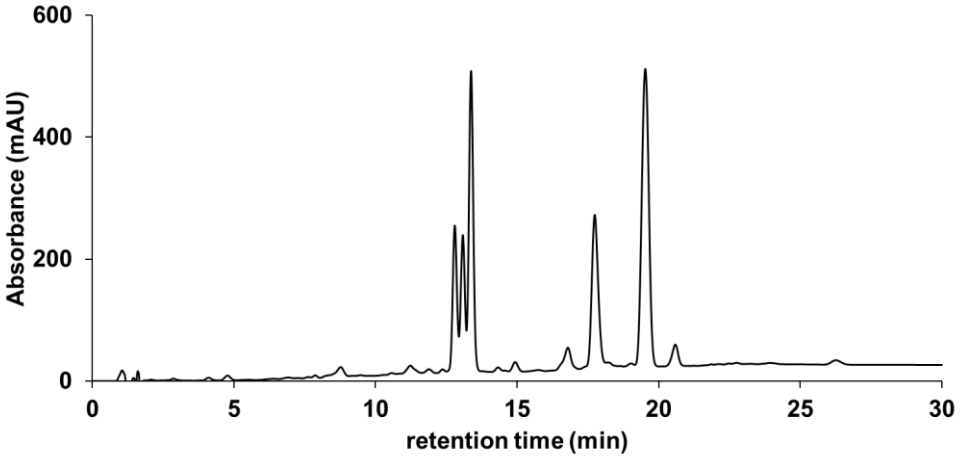
Cur6



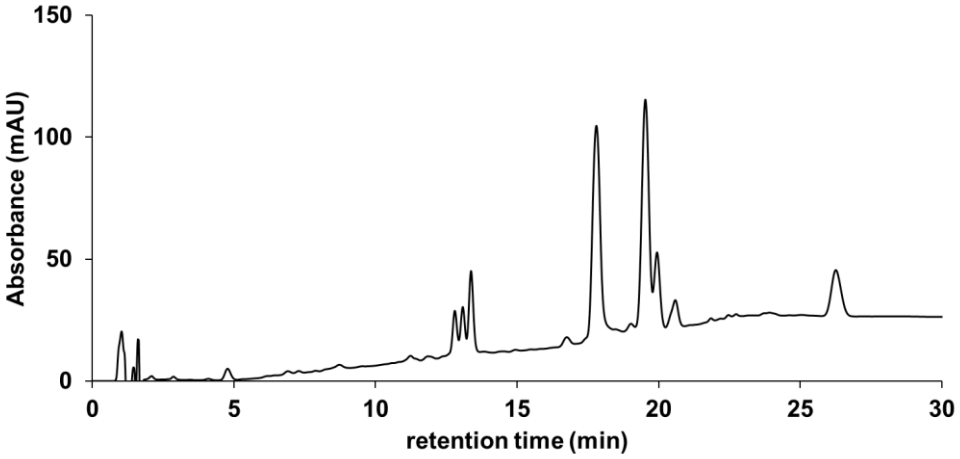
Cur7



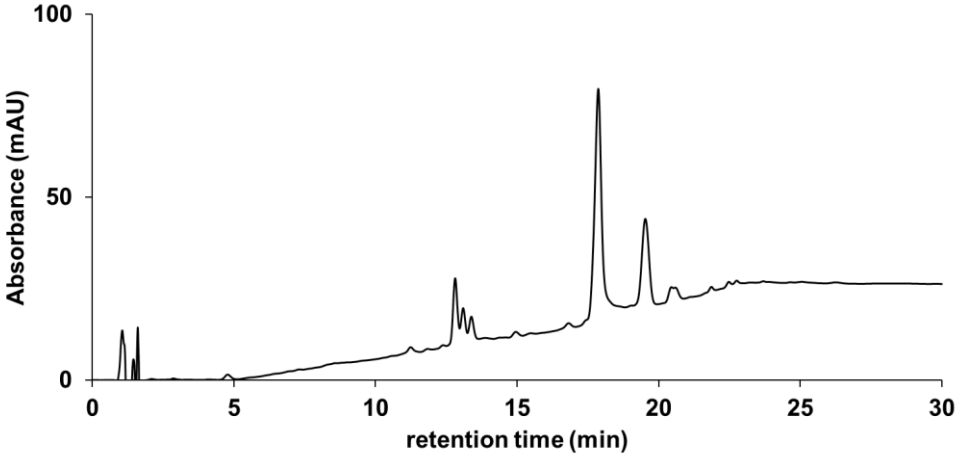
Cur8



Cur9



Cur10



Cur11

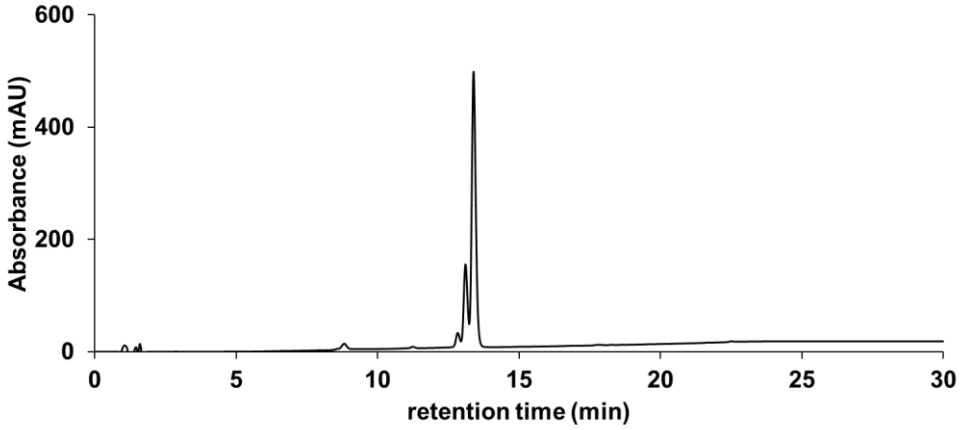
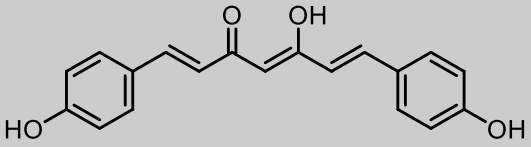
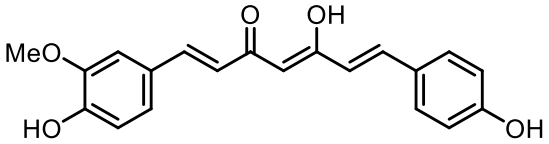
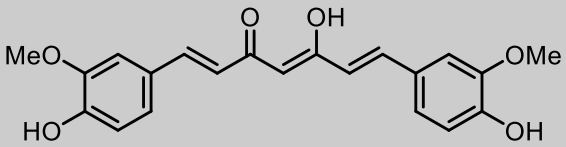
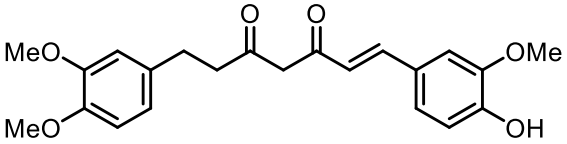
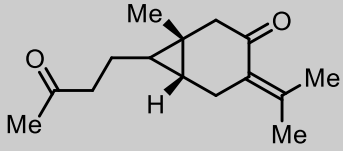
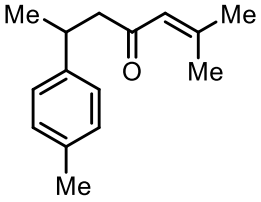
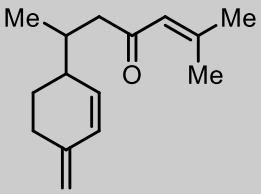

600
400
200
0
Absorbance (mAU)
0
5
10
15
20
25
30
retention time (min)

Table S3. Peak identifications of a methanol extract from **Cur2**.

Entry	Retention Time (min)	Theoretical m/z	Found m/z	Mass error (ppm)	Proposed Molecular Formula	Proposed structure
1	12.81	309.1121	309.1120	0.31	$C_{19}H_{16}O_4$	 bisdemethoxycurcumin
2	13.09	339.1227	339.1225	0.50	$C_{20}H_{18}O_5$	 demethoxycurcumin
3	13.38	369.1333	369.1329	0.90	$C_{21}H_{20}O_6$	 curcumin
4	14.97	385.1646	385.1649	-0.90	$C_{22}H_{24}O_6$	 compound A (or its regioisomers on -OMe groups or the double bond)
5	16.83	235.169	235.1691	-0.43	$C_{15}H_{22}O_2$	 curcumenone
6	17.77	217.1587	217.1585	0.90	$C_{15}H_{20}O$	 *ar*-turmerone
7	19.57	219.1740	219.1736	1.83	$C_{15}H_{22}O$	 β-turmerone

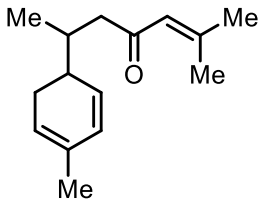
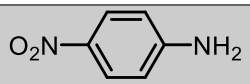
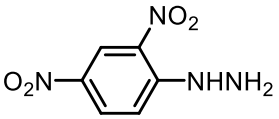
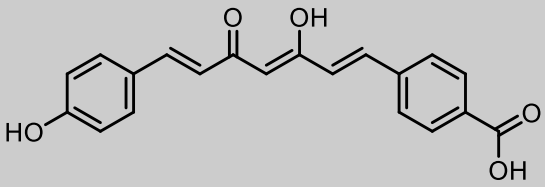
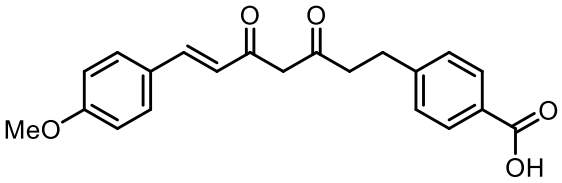
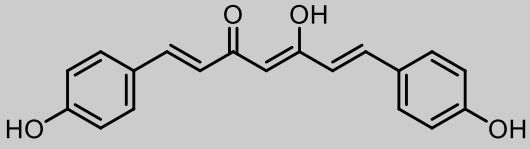
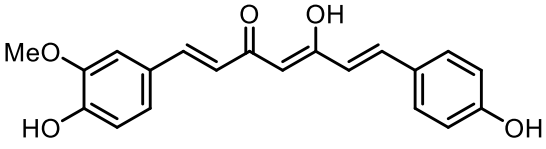
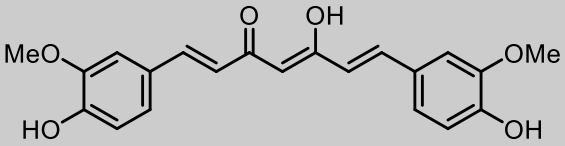
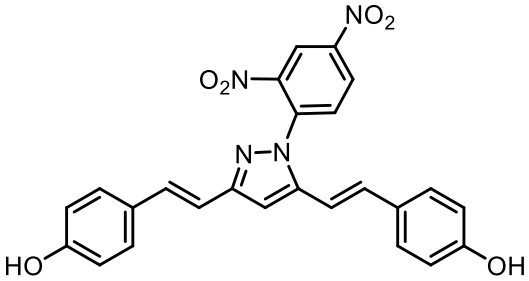
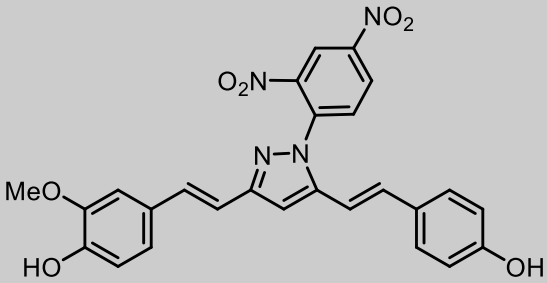
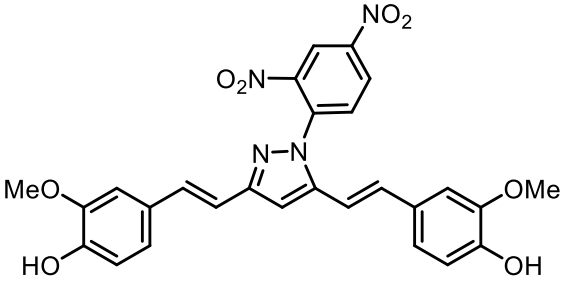
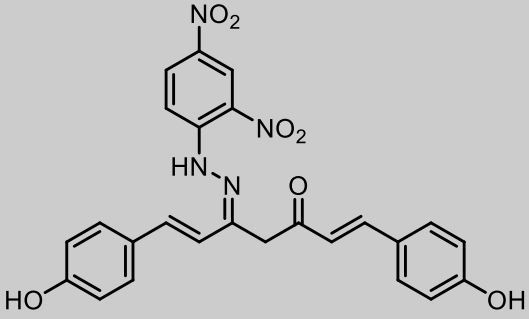
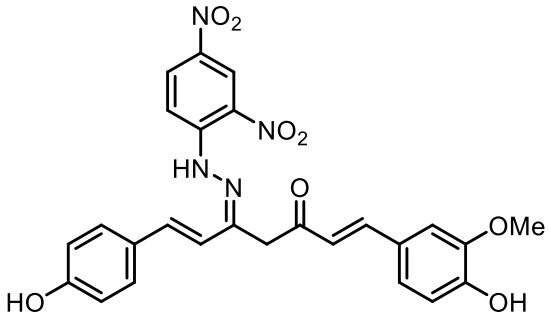
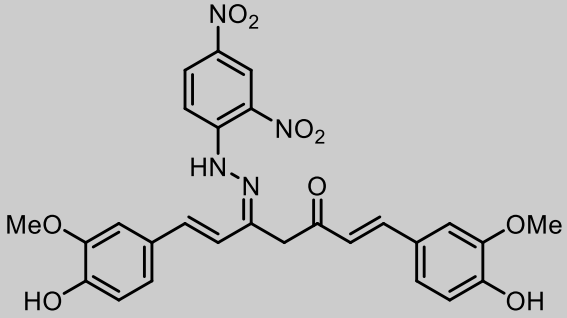
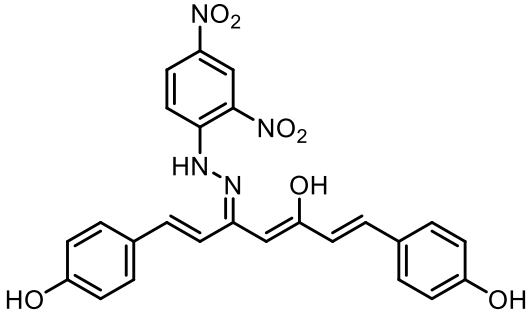
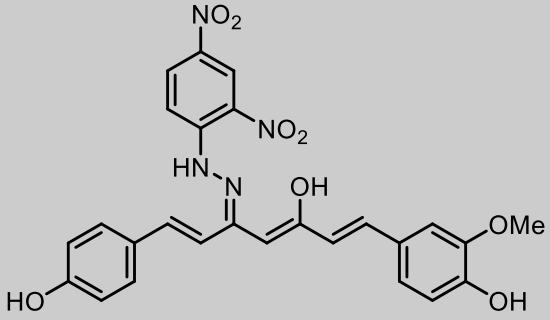
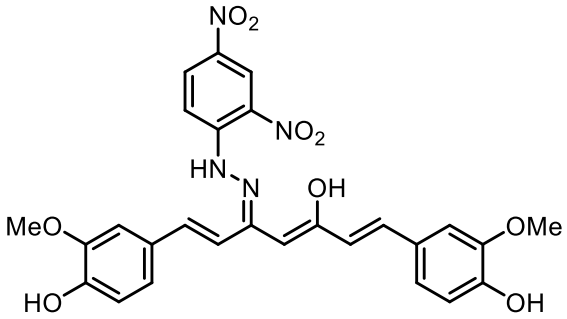
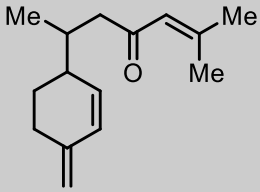
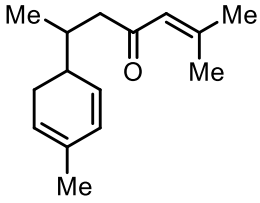
8	20.62	219.1740	219.1737	1.37	$C_{15}H_{22}O$	 α-turmerone

Table S4. Peak identifications of a methanol extract from **Cur2** after reacting with 2,4-DNP.

Entry	Retention Time (min)	Theoretical m/z	Found m/z	Mass error (ppm)	Proposed Molecular Formula	Proposed structure
1	7.33	139.0500	139.0501	-0.72	$C_6H_6N_2O_2$	 *p*-nitroaniline
2	8.35	199.0460	199.0460	0.00	$C_6H_6N_4O_4$	 2,4-DNP
3	9.51	337.1071	337.1070	0.15	$C_{20}H_{16}O_5$	 compound B (or other structural isomers)
4	11.58	353.1384	353.1382	0.42	$C_{21}H_{20}O_5$	 compound C (or other structural isomers)
5	12.85	309.1121	309.1118	0.98	$C_{19}H_{16}O_4$	 bisdemethoxycurcumin
6	13.13	339.1227	339.1225	0.69	$C_{20}H_{18}O_5$	 demethoxycurcumin

7	13.41	369.1333	369.1330	0.82	$C_{21}H_{20}O_6$	 curcumin
8	14.10	471.1300	471.1295	1.06	$C_{25}H_{18}N_4O_6$	 The condensed hydrazone product of bisdemethoxycurcumin
9	14.38	501.1400	501.1403	-0.60	$C_{26}H_{20}N_4O_7$	 The condensed hydrazone product of demethoxycurcumin (or its structural isomers)
10	14.62	531.1510	531.1505	0.94	$C_{27}H_{22}N_4O_8$	 The condensed hydrazone product of curcumin
11	16.63	489.1400	489.1409	-1.84	$C_{25}H_{20}N_4O_7$	 The hydrazone product of bisdemethoxycurcumin (keto)

12	16.63	519.1510	519.1514	-0.77	$C_{26}H_{22}N_4O_8$	 The hydrazone product of demethoxycurcumin (keto) (or its structural isomers)
13	16.63	549.1620	549.1611	1.64	$C_{27}H_{24}N_4O_9$	 The hydrazone product of curcumin (keto)
14	17.98	489.1400	489.1403	-0.61	$C_{25}H_{20}N_4O_7$	 The hydrazone product of bisdemethoxycurcumin
15	17.98	519.1510	519.1512	-0.39	$C_{26}H_{22}N_4O_8$	 The hydrazone product of demethoxycurcumin (or its structural isomers)

16	17.98	549.1620	549.1612	1.46	$C_{27}H_{24}N_4O_9$	 The hydrazone product of curcumin
17	19.61	219.1743	219.1740	1.56	$C_{15}H_{22}O$	 β-turmerone
18	20.65	219.1743	219.1737	2.93	$C_{15}H_{22}O$	 α-turmerone
19	22.44	415.1980	415.1977	0.72	$C_{21}H_{26}N_4O_5$	The hydrazone product of curcumenone
20	23.45	397.1870	397.1858	3.02	$C_{21}H_{24}N_4O_4$	The hydrazone product *ar*-turmerone
21	24.36	343.1401	343.1407	-1.80	$C_{17}H_{18}N_4O_4$	The hydrazone product of a ketone with a formula of $C_{11}H_{14}O$, *e.g.*, 4-methylphenyl propyl ketone.
22	25.28	399.2027	399.2018	2.20	$C_{21}H_{26}N_4O_4$	The hydrazone product of β-turmerone

Preparation of reagents in the array

The following section is the list of the reagents used in this study with their concentrations.

- pH 2 = 5 μL of 100 mM pH 2 phosphate buffer in Milli-Q water.
- pH 7 = 5 μL of 100 mM pH 7 phosphate buffer in Milli-Q water.
- pH 12 = 5 μL of 100 mM pH 12 phosphate buffer in Milli-Q water.
- 2,4-DNP = 5 μL of 4 mM 2,4-DNP solution (prepared by diluting a stock 0.2-M 2,4-DNP in H_2SO_4: H_2O: MeOH solution (4:10:35) in Milli-Q water at 1:50 dilution)
  The stock solution of 2,4-DNP was prepared by the following methods (J. Brady, *J. Chem. Soc*. **1931**, 756):
  Add 2 g of 2,4-dinitrophenylhydrazine (0.2 M) into 4 mL of concentrated sulfuric acid and cautiously add 35 mL of methanol with cooling. Warm solution at 50 °C for 1 hour and then add 10 mL of water. Keep the solution at room temperature for 2 hour and filter insoluble solid out.
- Vanillin = 5 μL of 6.6-mM vanillin solution (prepared by diluting a stock 66-mM vanillin in 70% aqueous sulfuric acid in Milli-Q water at 1:10 dilution).
- H_3BO_3 = 5 μL of 5-mM $Na_2B_4O_7$ solution (prepared by diluting a stock 50-mM $Na_2B_4O_7$ in 30% aqueous sulfuric acid in Milli-Q water at 1:10 dilution).
- $CuSO_4$ = 5 μL of 1 mM $CuSO_4.5H_2O$ in Milli-Q water
- $FeSO_4$ = 5 μL of 1 mM $FeSO_4.7H_2O$ in Milli-Q water
- $NiSO_4$ = 5 μL of 10 mM $NiSO_4.6H_2O$ in Milli-Q water
- $Pb(NO_3)_2$ = 5 μL of 10 mM $Pb(NO_3)_2$ in Milli-Q water

Intensities as numerical values of responses of paper arrays to chemical reagents

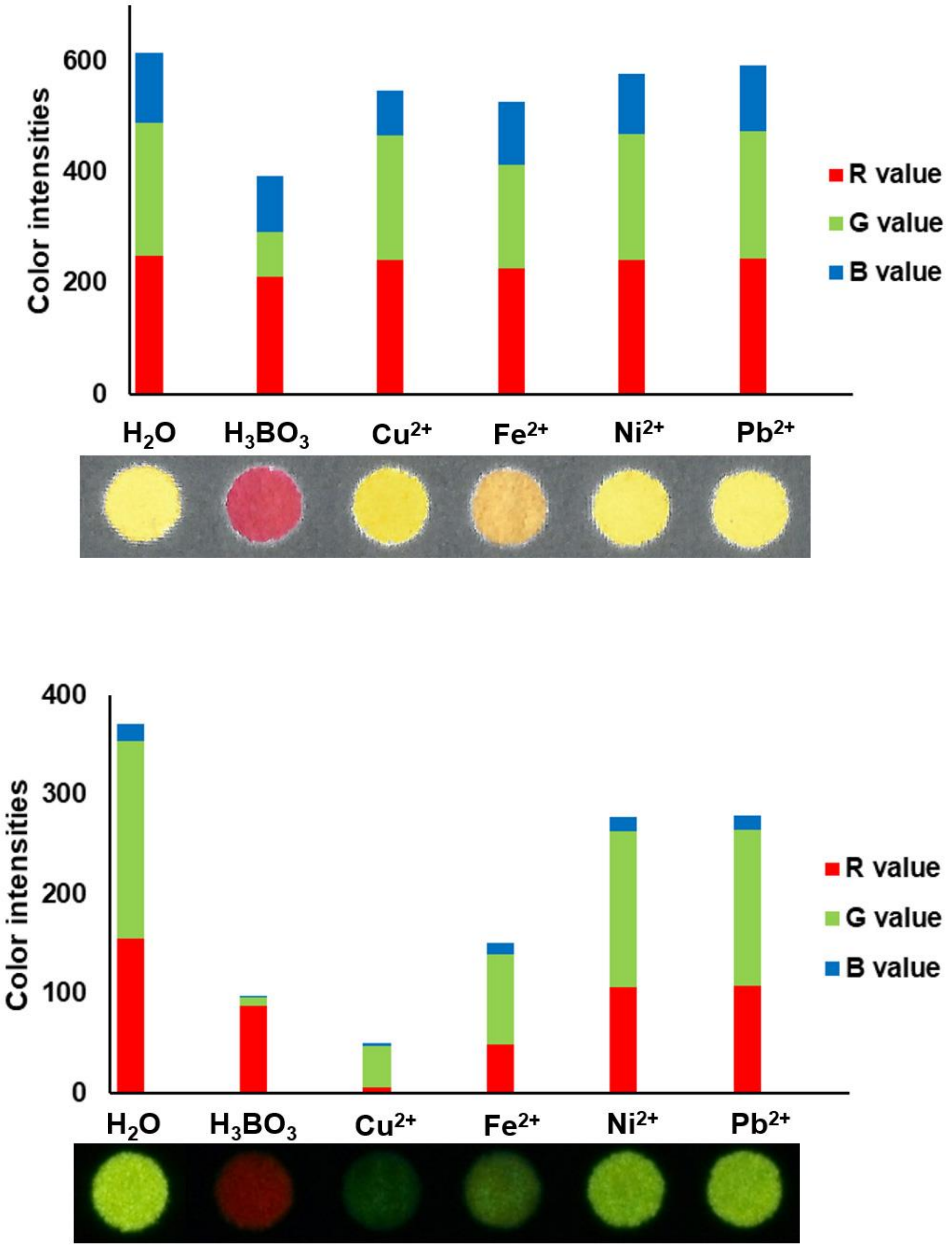
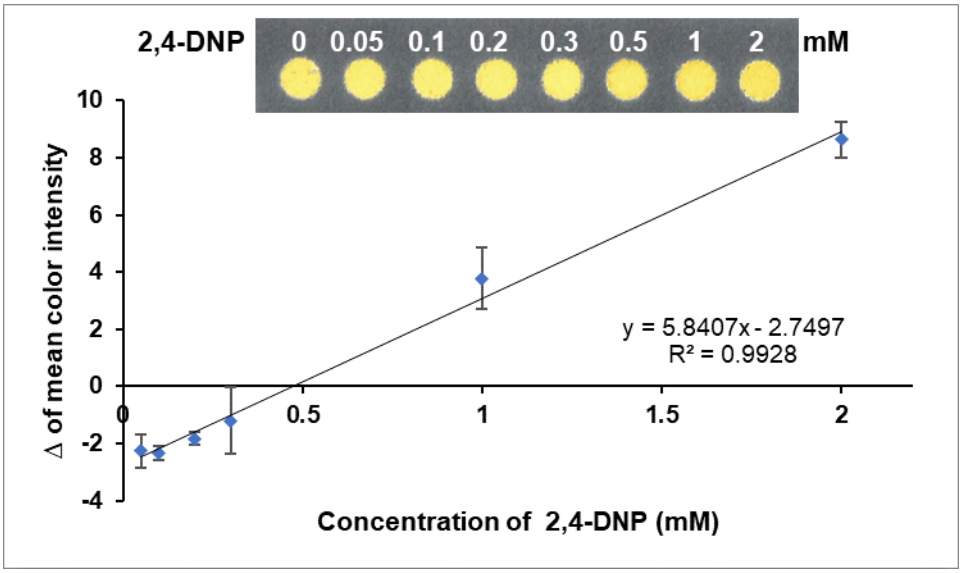
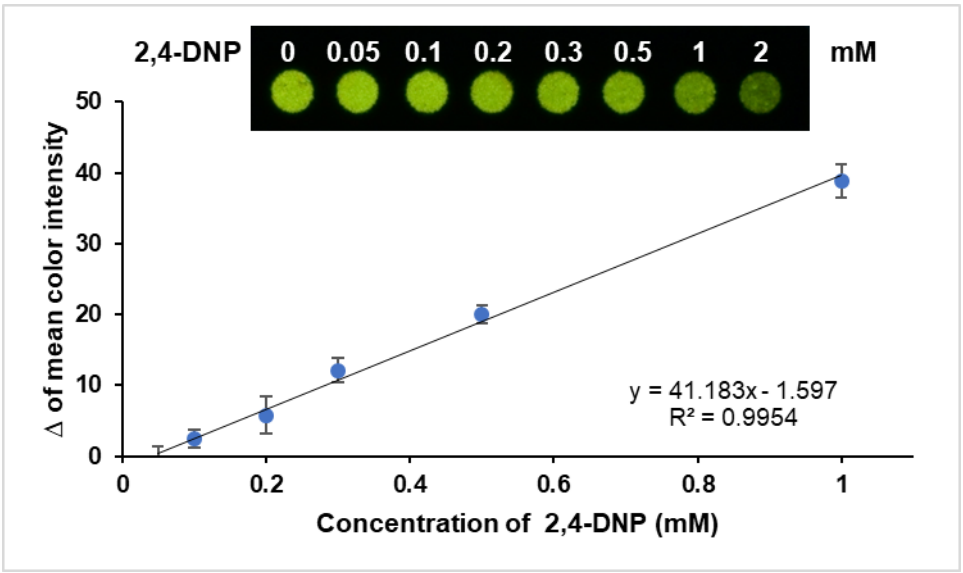


Figure S1. Colorimetric and fluorescence responses of paper arrays to various reagents (native concentrations as shown in the preparation section) after being exposed to 0.2-mM curcumin standard under white light (top) and 365-nm UV light (bottom). The values for all RGB channels were also shown.

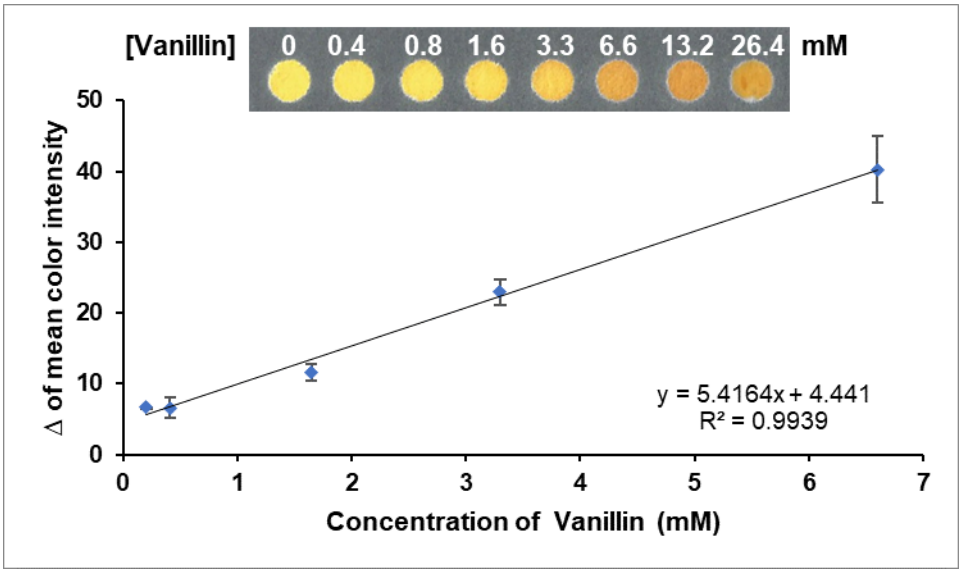


Limit of detection = 1.3 x 10^{-4} M

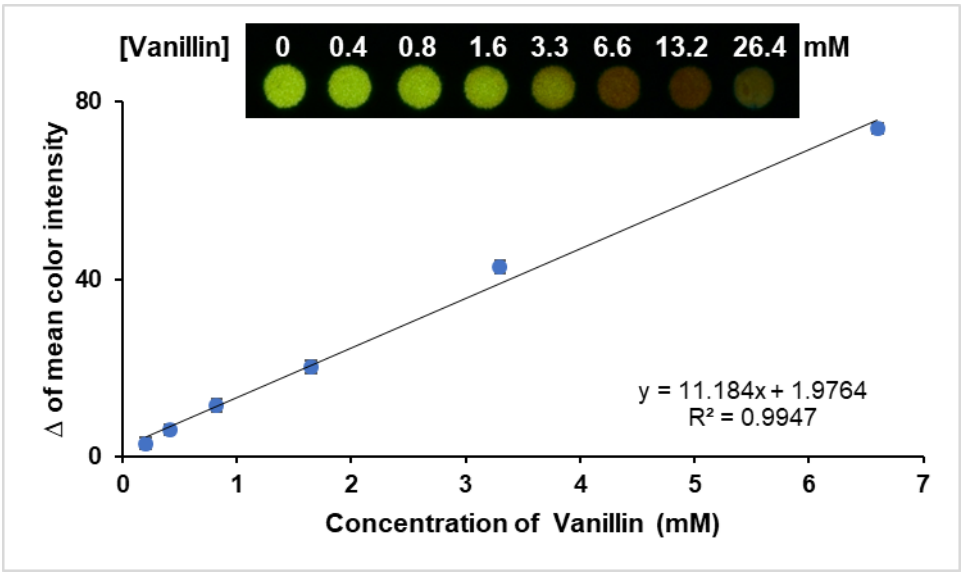


Limit of detection = 5.4 x 10^{-5} M

Figure S2. Colorimetric and fluorescence responses of 0.2-mM standard 98% curcumin with different concentrations of 2,4-DNP and calibration plots of the Δ of mean color intensity (n = 3) under white light (top) and black light (bottom).

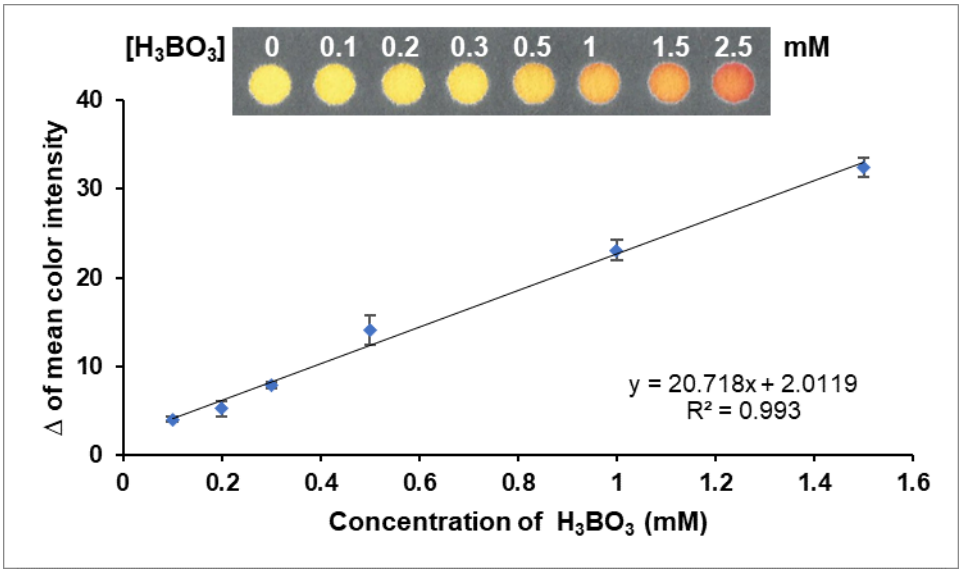


Limit of detection = 6.3 x 10^{-4} M

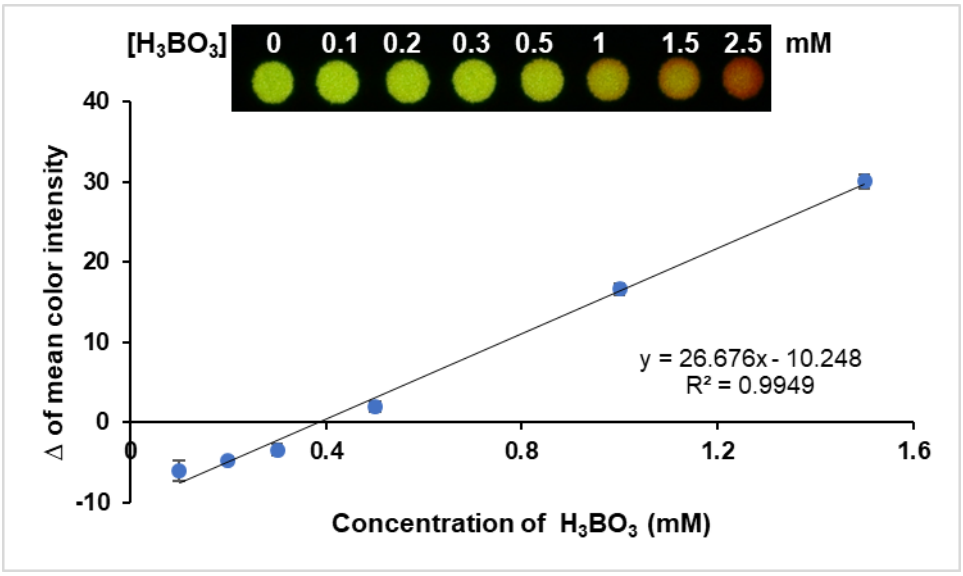


Limit of detection = 3.7 x 10^{-4} M

Figure S3. Colorimetric and fluorescence responses of 0.2-mM standard 98% curcumin with different concentrations of vanillin and calibration plots of the Δ of mean color intensity (n = 3) under white light (top) and black light (bottom).

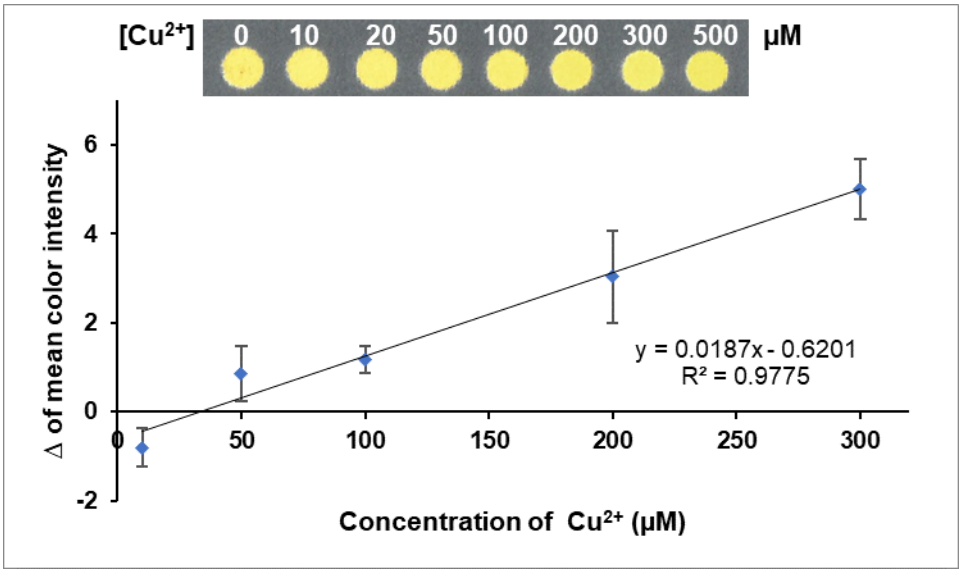


Limit of detection = 1.1 x 10^{-4} M

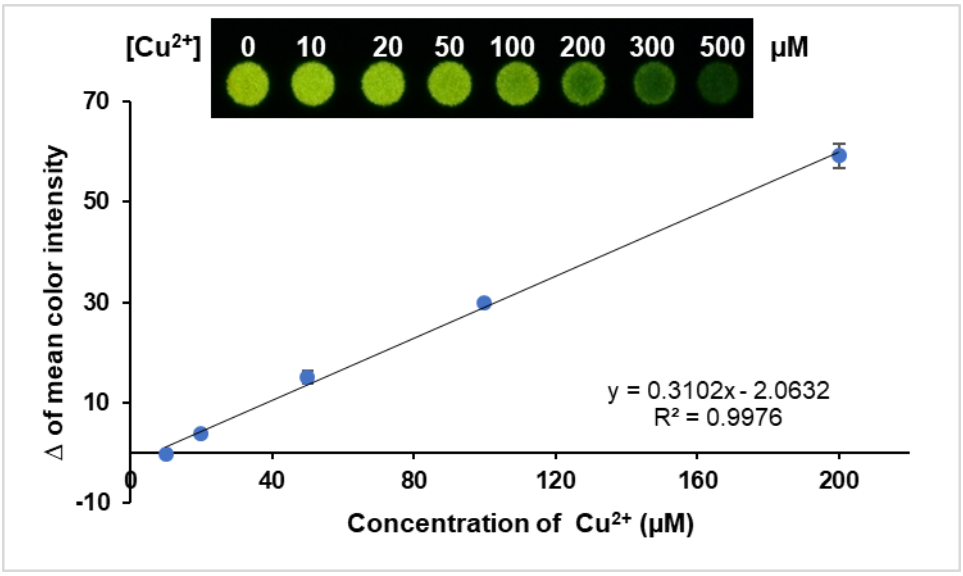


Limit of detection = 9.2 x 10^{-5} M

Figure S4. Colorimetric and fluorescence responses of 0.2-mM standard 98% curcumin with different concentrations of H_3BO_3 and calibration plots of the Δ of mean color intensity (n = 3) under white light (top) and black light (bottom).

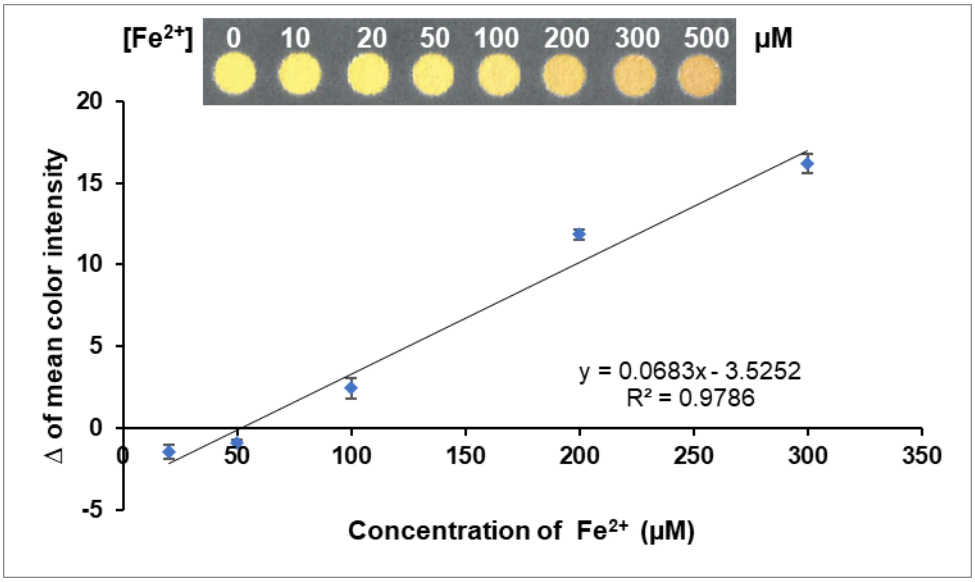


Limit of detection = 4.9 x 10^{-5} M

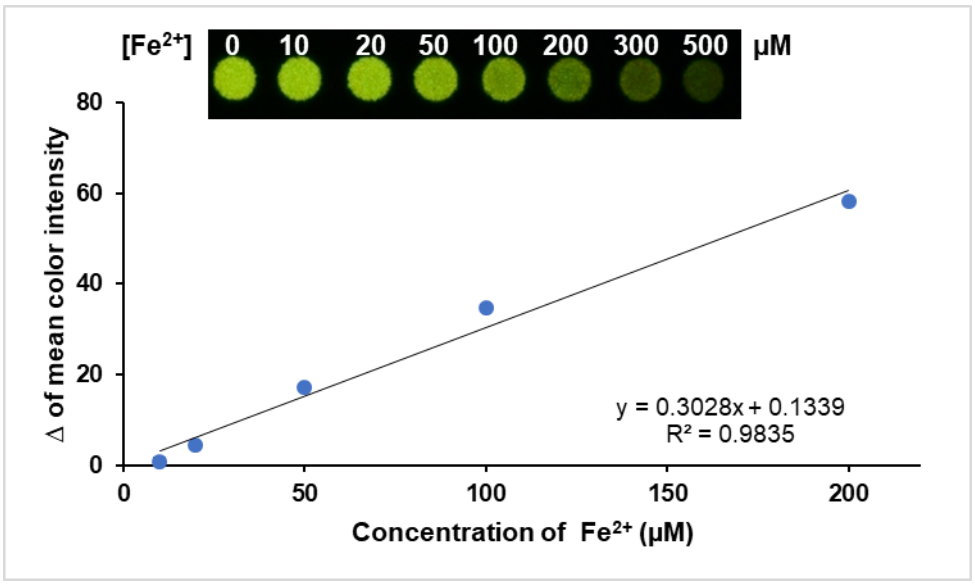


Limit of detection = 9.6 x 10^{-6} M

Figure S5. Colorimetric and fluorescence responses of 0.2-mM standard 98% curcumin with different concentrations of Cu^{2+} and calibration plots of the Δ of mean color intensity (n = 3) under white light (top) and black light (bottom).



Limit of detection = 4.8 x 10^{-5} M



Limit of detection = 2.5 x 10^{-5} M

Figure S6. Colorimetric and fluorescence responses of 0.2-mM standard 98% curcumin with different concentrations of Fe^{2+} and calibration plots of the Δ of mean color intensity (n = 3) under white light (top) and black light (bottom).

Chemometric protocol

LDA computation involves two main parts. First, all combinations of factors and variables were calculated based on binary numberal system. This involves all possible combination of *n* elements equal to 2^n-1 (the combination of all zeros was ignored).

```
%Function for create all combinatorial of factors/variables
%This function was created based on binary numberal system
%It will create number of possible cominations (2^n)-1
%Where n equal to elements/factors/variables
%Where row represents respective combinations (2^n-1)
%and column represents each element (n)
%Input : only n %Output : output matrix contain
function combi=totcombi(n)
%This part creates all combinations of elements
a=2^(n);
for i=1:(a-1)
    b=dec2bin(i);
    c=[zeros(1,n-size(b,2)),b];
    for j=1:n
        if c(j)=='1'
            d(i,j)=n-j+1;
        else
            d(i,j)=0;
        end
    end
end
%rearrange in form of matrix
for i=1:(a-1)
    u0=unique(d(i,:));
    u=u0(1,u0~=0);
    nu(i)=size(u,2);
end
combi=zeros(1,n);
for i=1:n
    for j=1:(a-1)
        r=d(nu==i,:);
    end
    combi=[combi;r];
end
combi(1,:)=[];
combi=fliplr(combi);
end
```

An example of output from this function: If dataset with 3 elements are considered, it will involve 2^3-1 = 7 combinations as follows:

	Element 1	**Element 2**	**Element 3**
Combination 1	**1**	**0**	**0**
Combination 2	**0**	**2**	**0**
Combination 3	**0**	**0**	**3**
Combination 4	**1**	**2**	**0**
Combination 5	**1**	**0**	**3**
Combination 6	**0**	**2**	**3**
Combination 7	**1**	**2**	**3**

*0: not selected for further calculation;

Second, the dataset with only selected elements was used to for further calculation based on LDA approach. Briefly, the distance between samples to the class centroid is weighted according to the overall variance of each variable/element. The class of sample is determined as the class that provides the smallest distance to the sample.

```
% LDACLASSIFY Classifies test set samples using Linear Discriminant Analysis
%
%   TESTY = LDACLASSIFY_Multi(TRAINX, TRAINY, TESTX)
%   INPUTS
%       trainX = i x j training set data matrix (i = samples, j =
%            variables).
%       trainY = i x 1 class vector of samples in the training set.
%       testX  = k x j test set sample matrix; the samples to be classified.
%
%   OUTPUTS
%       testY  = k x 1 predicted class vector for the test set samples
%       dist   = k x 2 distances (squared) of sample to each class

% Implemented by K.Wongravee & G.R.Lloyd
% Reference : R.G. Brereton, "Chemometrics for pattern recognitions",
%            Wiley, Chichester, 2009 (Section 5.3)

function  [testY,dist]=LDAClassify_Multi(trainX,trainY,testX)

u=unique(trainY);
trainXA = trainX(trainY==u(1),:);
trainXB = trainX(trainY==u(2),:);
CP=zeros(size(trainX,2));
for i=1:length(u)
    C=cov(trainX(trainY==u(i),:),1);
    M(i,:)=mean(trainX(trainY==u(i),:));
    N(i,1)=size(trainX(trainY==u(i),:),1);
    CP = CP + ((N(i,1)-1)*C);
end
CP=inv(CP./sum(N-1));
dist=zeros(size(testX,1),length(u));
for i=1:size(testX,1)
    for j=1:length(u)
        dist(i,j)=(testX(i,:)-M(j,:))*(CP)*(testX(i,:)-M(j,:))';
    end
end
[m,testY]=min(dist,[],2);
```

Readers can contact Assist. Prof. Dr. Kanet Wongravee (Kanet.W@chula.ac.th) for further questions.

Additional chemometric data

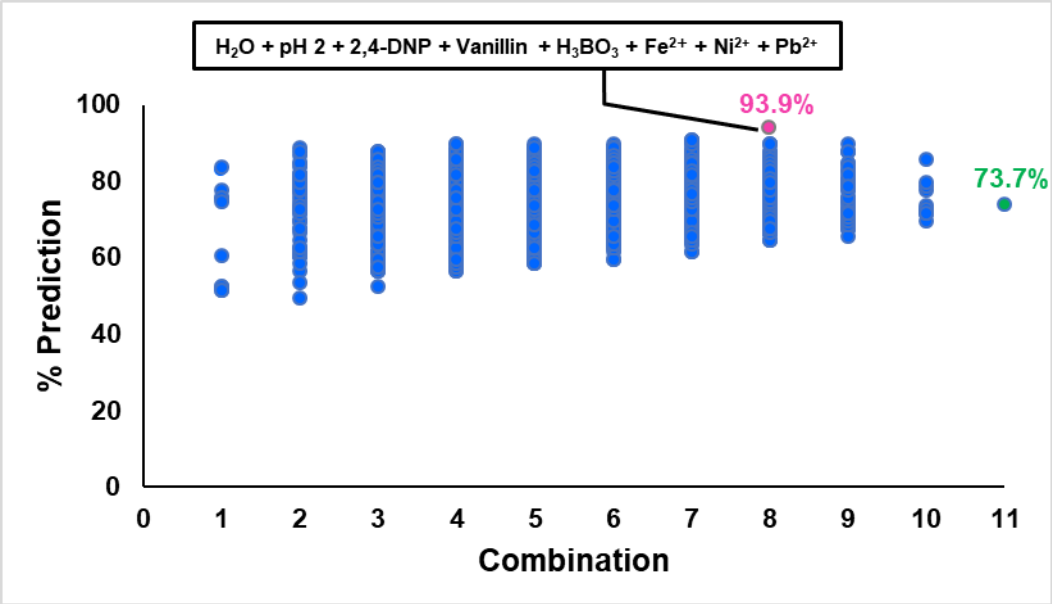


Figure S7. A plot between the percentages of prediction accuracies vs the numbers of reagents used in the differentiation process.