Extraction and analysis of bioactive compounds from fresh and dried food products

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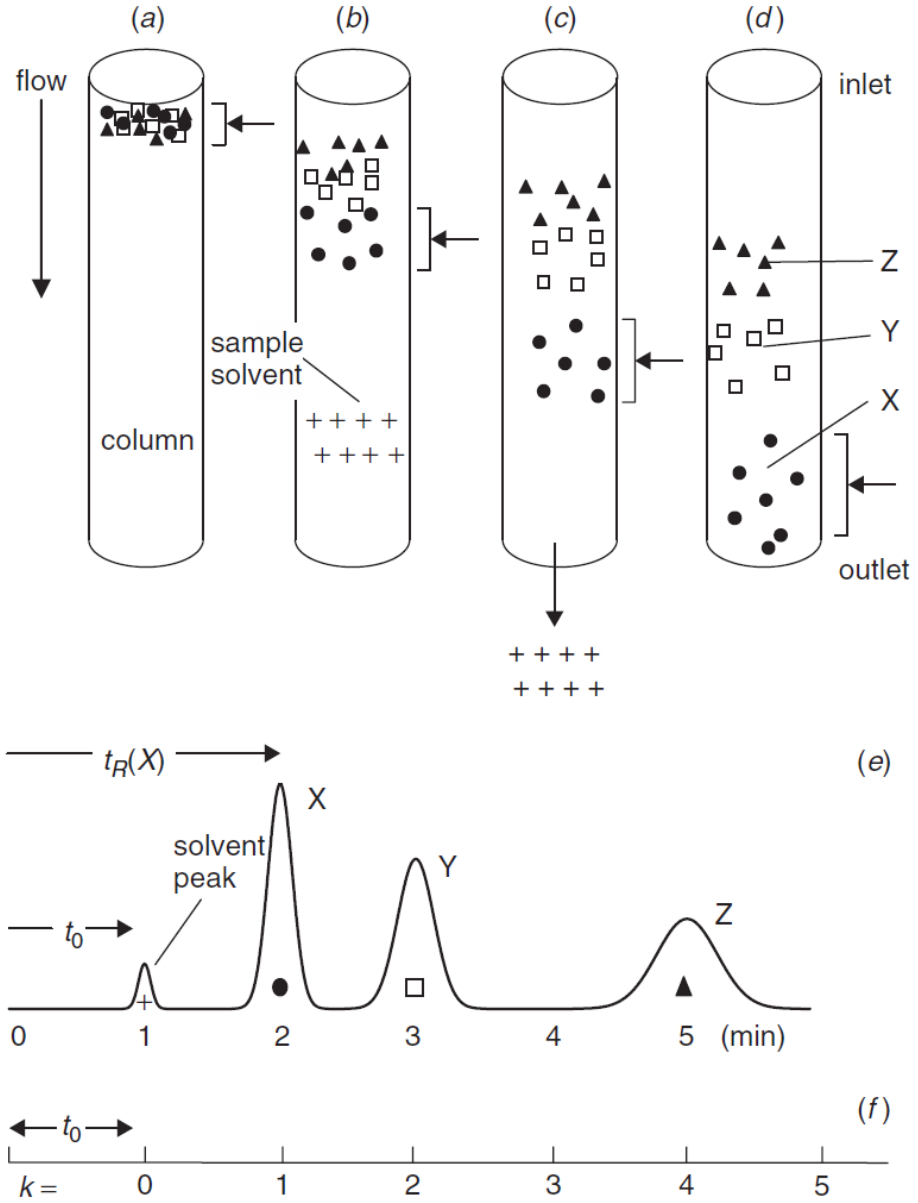
Topics

- High Performance liquid chromatography (HPLC)
- Antioxidant capacity using DPPH assay
- Total phenolic content using Folin-Ciocalteu reagent

HPLC

- Fundamentals of chromatography
- HPLC instrument
- Total phenolic content using Folin-Ciocalteu reagent

HPLC for bioactive compounds analysis



Fundamentals of chromatography for separation of compounds

Snyder, L. R.; Kirkland, J. J.; Dolan, J. W., *Introduction to Modern Liquid Chromatography. John Wiley & Sons: 2010; p 912.*

HPLC basic components

- Pump → high pressure, high accuracy pump
- Injector → manual, autosampler
- Column → a lot of types
- Detector → (UV, RI,ELSD, EC, MS)
- PC/Recorder

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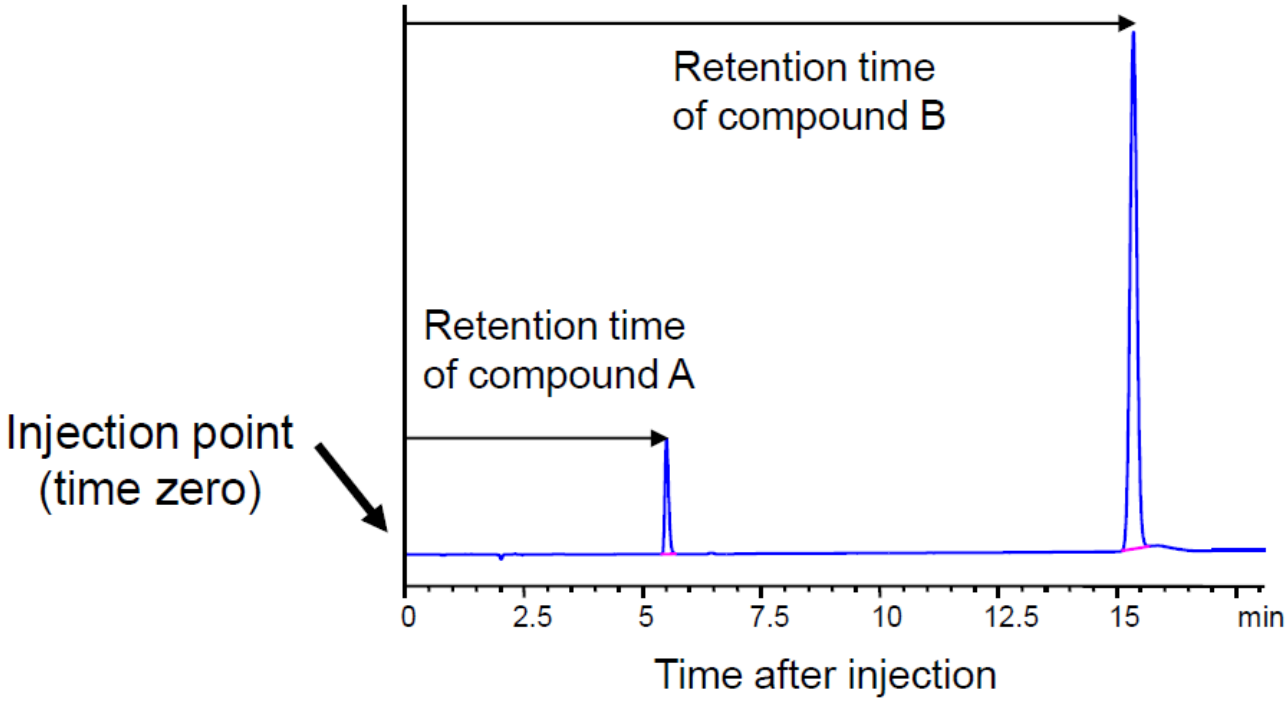
HPLC utilization

- Identify compounds



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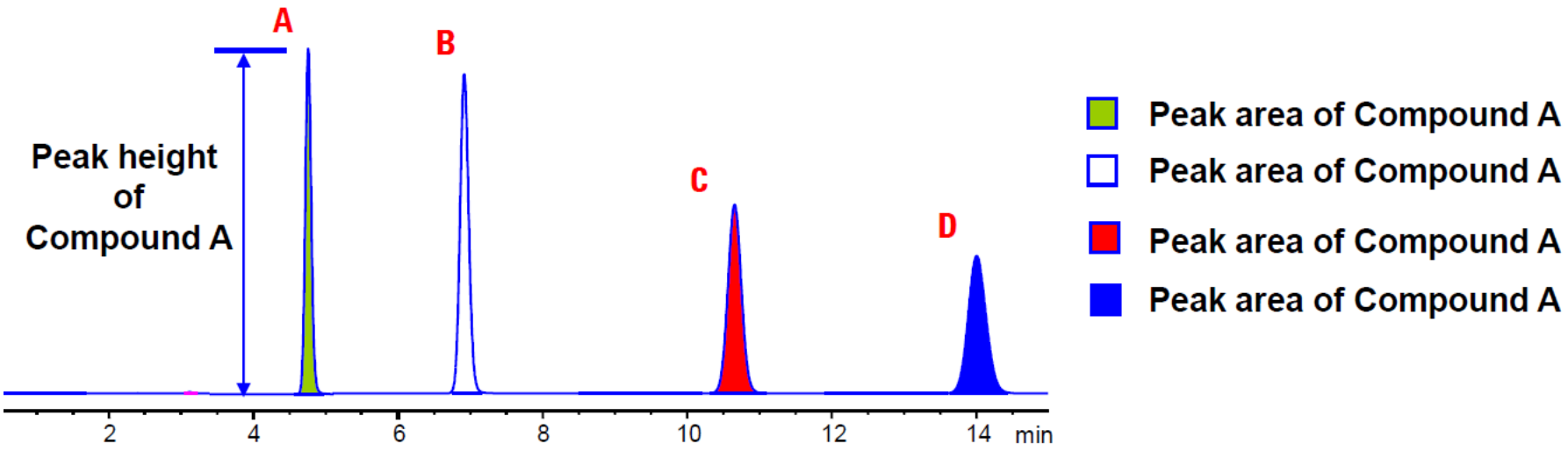
HPLC utilization

- Quantify compounds



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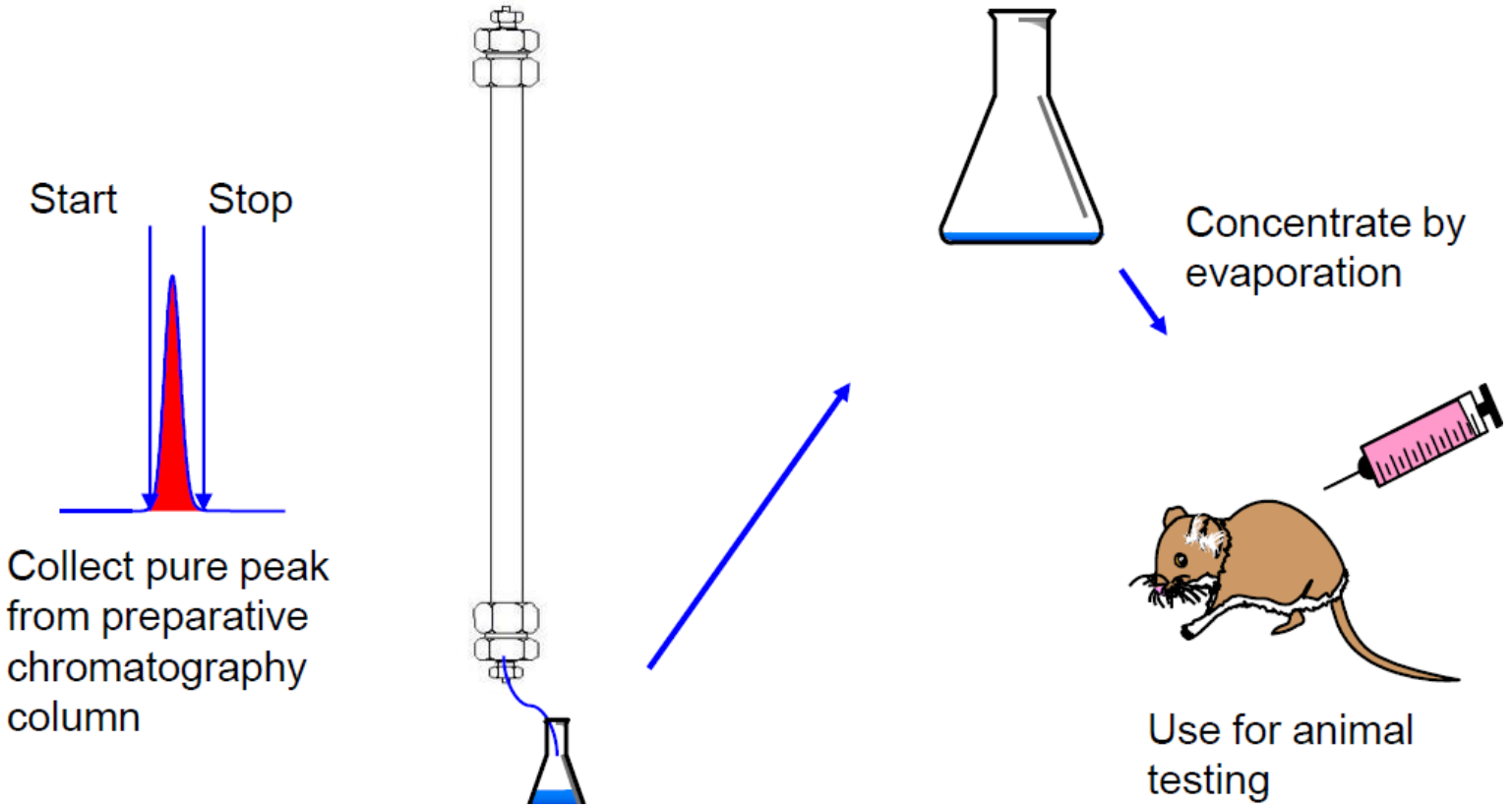
HPLC utilization

- Preparation of pure substance



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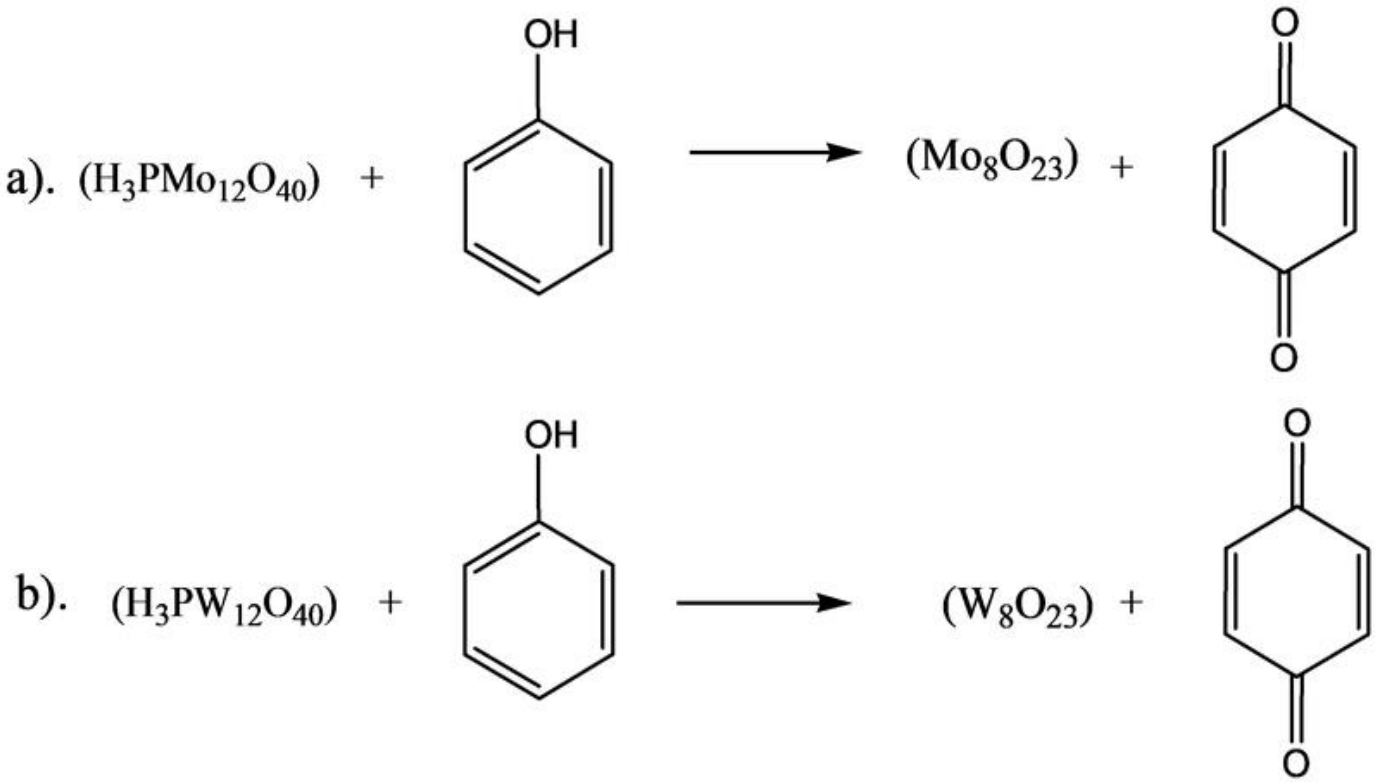
Total phenolic content

- Total phenolic content (TPC) using Folin-Ciocalteu reagent is a widely employed method for rough estimating of an amount of phenolic compounds
- Developed by Otto Folin and Vintila Ciocalteu since 1927
- Phosphomolybdic acid ($H_3PMo_{12}O_{40}$)/ phosphotungstic acid ($H_3PW_{12}O_{40}$) is reduced by phenolic hydroxyl in alkaline solution (pH ~10) to form tungsten and molybdenum blue



- The dye can be measured at 760 nm.
- Gallic acid is often used as reference and the result is expressed as “mg gallic acid equivalent (GAE)”

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In Methods in Enzymology (Vol. 299, pp. 152-178): Academic Press

Interference

- compounds other than phenolics, such as reducing sugars (e.g., glucose, fructose, maltose) and ascorbic acid, are also able to reduce the FC reagent
- We found that Maillard reaction products also react with FC, and this might be affect the results of TPC in dried products (unpublished data)

Table 1. Reactivity of Various Substances with Folin−Ciocalteu Phenol Reagent

compound	molar mass (g/mol)	GAE (mass)	GAE (molar)
phenolic compounds			
caffeic acid	180.16	1.00	0.958
chlorogenic acid	354.31	0.722	1.36
curcumin	368.40	0.722	1.41
ellagic acid	302.19	1.32	2.12
ferulic acid	194.18	1.05	1.08
gallic acid	188.14	1.00	1.00
quercetin	338.00	1.16	2.08
resveratrol	228.25	1.01	1.23
rutin	610.52	0.568	1.53
salicylic acid	138.12	0.357	0.262
tannic acid	1701.00	0.878	9.04
thiol derivatives			
amifostine	214.22	0.378	0.430
captopril	217.29	0.323	0.373
cysteamine HCl	113.61	0.304	0.184
glutathione	307.30	0.161	0.263
MPG	163.20	0.342	0.297
N-acetylcysteine	163.20	0.395	0.378
penicillamine	149.21	0.333	0.264
PTCA	175.25	0.180	0.141
RibCys	253.23	0.202	0.271
WR-1065	134.24	0.375	0.268
vitamin derivatives			
D-sucrose	342.30	0.000	0.000
egg albumin	~65 kDa	0.0163	5.63
bovine serum albumin	69.3 kDa	0.0282	10.39
aldehydes, ketones, and carboxylic acids			
cinnamic acid	148.17	0.000	0.000
citric acid	192.12	0.000	0.000
oxalic acid	126.07	0.000	0.000
quinic acid	197.17	0.000	0.000
sodium tartrate	196.07	0.000	0.000
α-ionone	192.30	0.0043	0.0044
2,3-butanedione	86.09	0.180	0.00824
cinnamaldehyde	132.16	0.000	0.000
citronellal	154.25	0.000	0.000
inorganic salts			
iron(II) chloride	126.73	0.149	0.100
manganese(II) chloride	125.84	0.0432	0.0289
sodium nitrite	85.01	0.000	0.000
sodium sulfite	126.04	0.0506	0.0339
potassium iodide	166.00	0.0224	0.0198
miscellaneous compounds			
caffeine	194.19	0.000	0.000
cystamine[a]	225.20	0.000	0.000
glutathione disulfide	612.60	0.000	0.000
menthol	156.27	0.000	0.000

Everette et al. suggested that FC assay should be viewed as antioxidant capacity rather than phenolic compound content

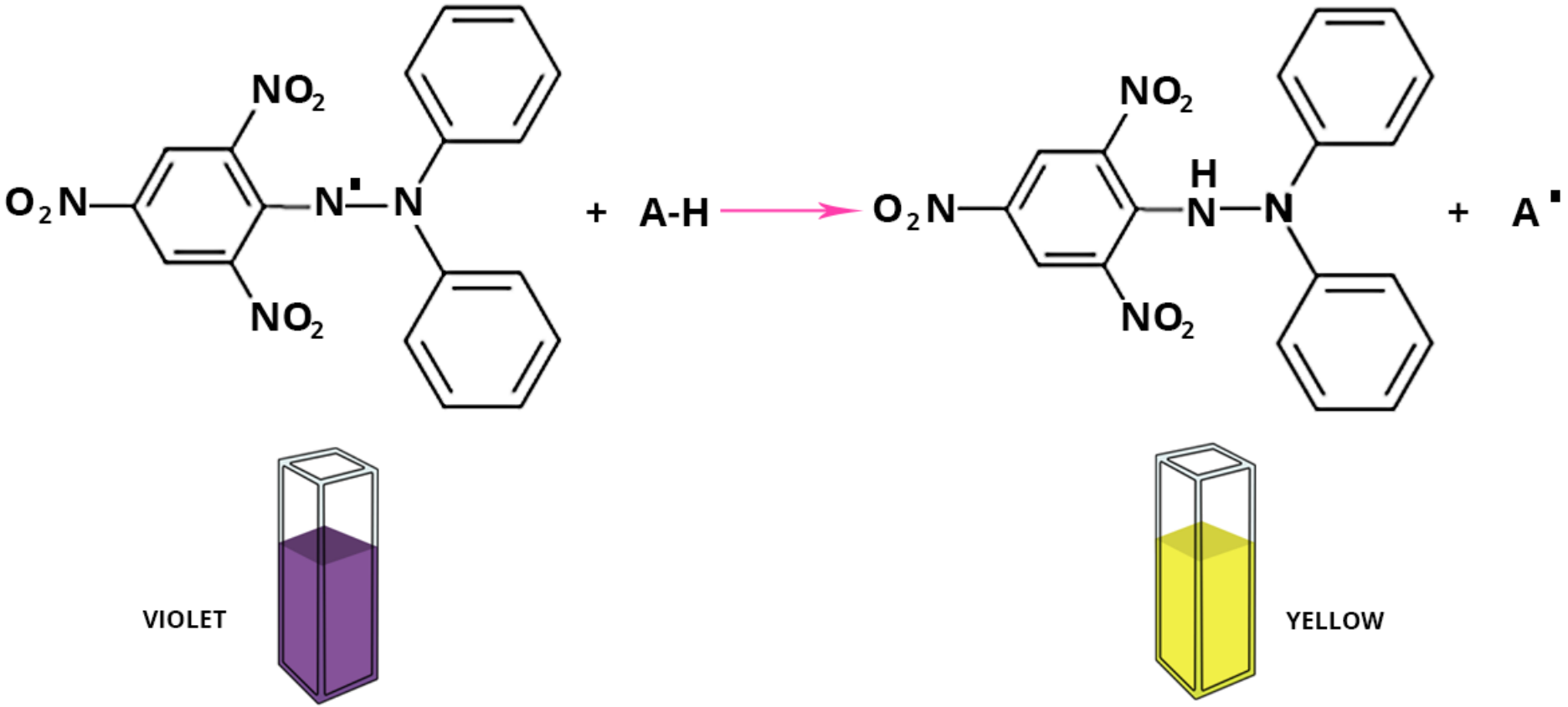
https://doi.org/10.1021/jf1005935

DPPH assay

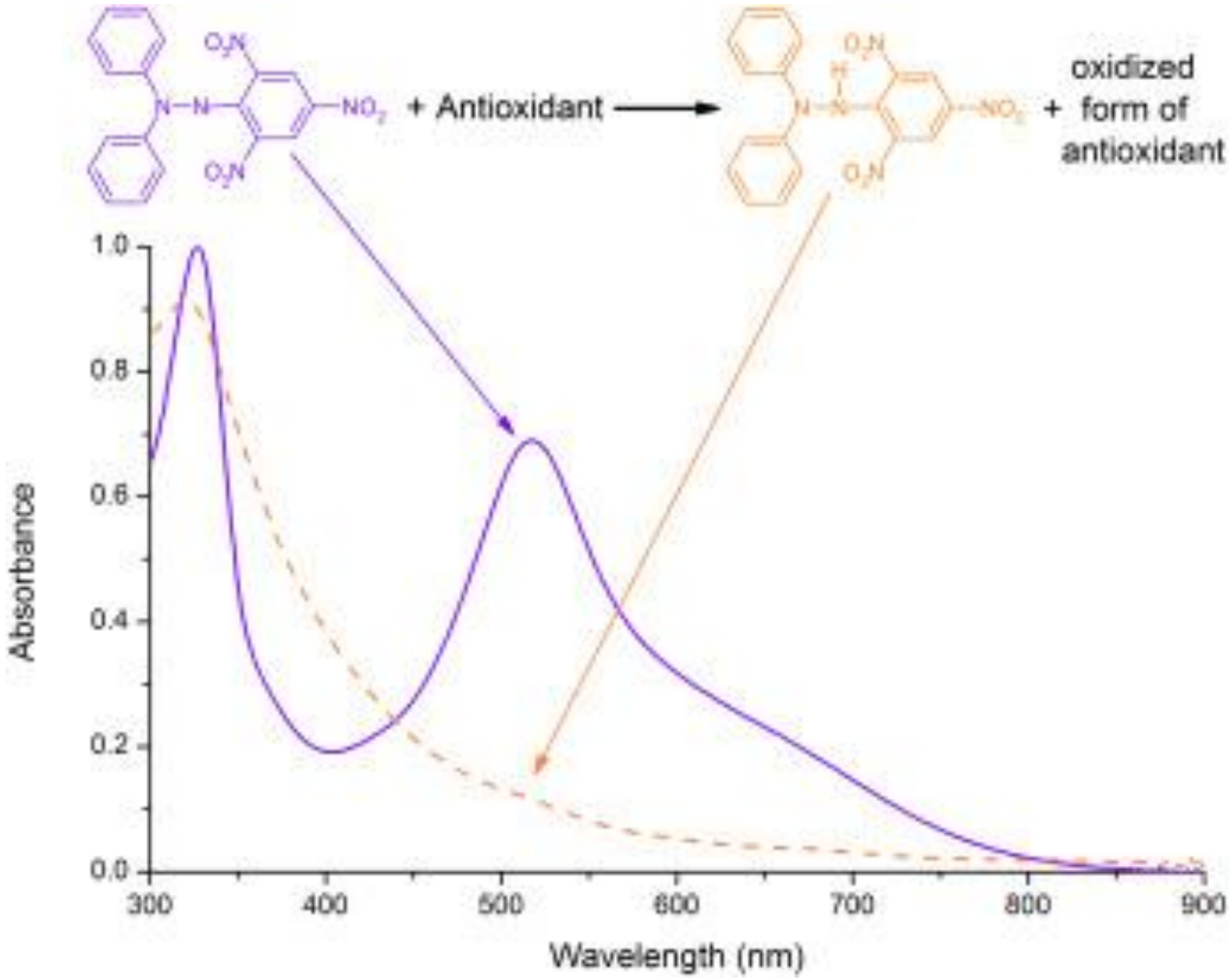
- Easy, economic and rapid method to evaluate the radical scavenging activity of substance
- 2,2-Diphenyil-picrylhydrazyl (DPPH) is a stable radical possesses a purple color, with a maximum absorption at around 515-519 nm in solvent such as methanol or ethanol
- Scavenging DPPH by antioxidant will reduce the absorbance

Radical form

Non-Radical form



http://chimactiv.agroparistech.fr/en/aliments/antioxydant-dpph/principe


+ Antioxidant
oxidized
+ form of
antioxidant
Absorbance
1.0
0.8
0.6
0.4
0.2
0.0
300
400
500
600
700
800
900
Wavelength (nm)

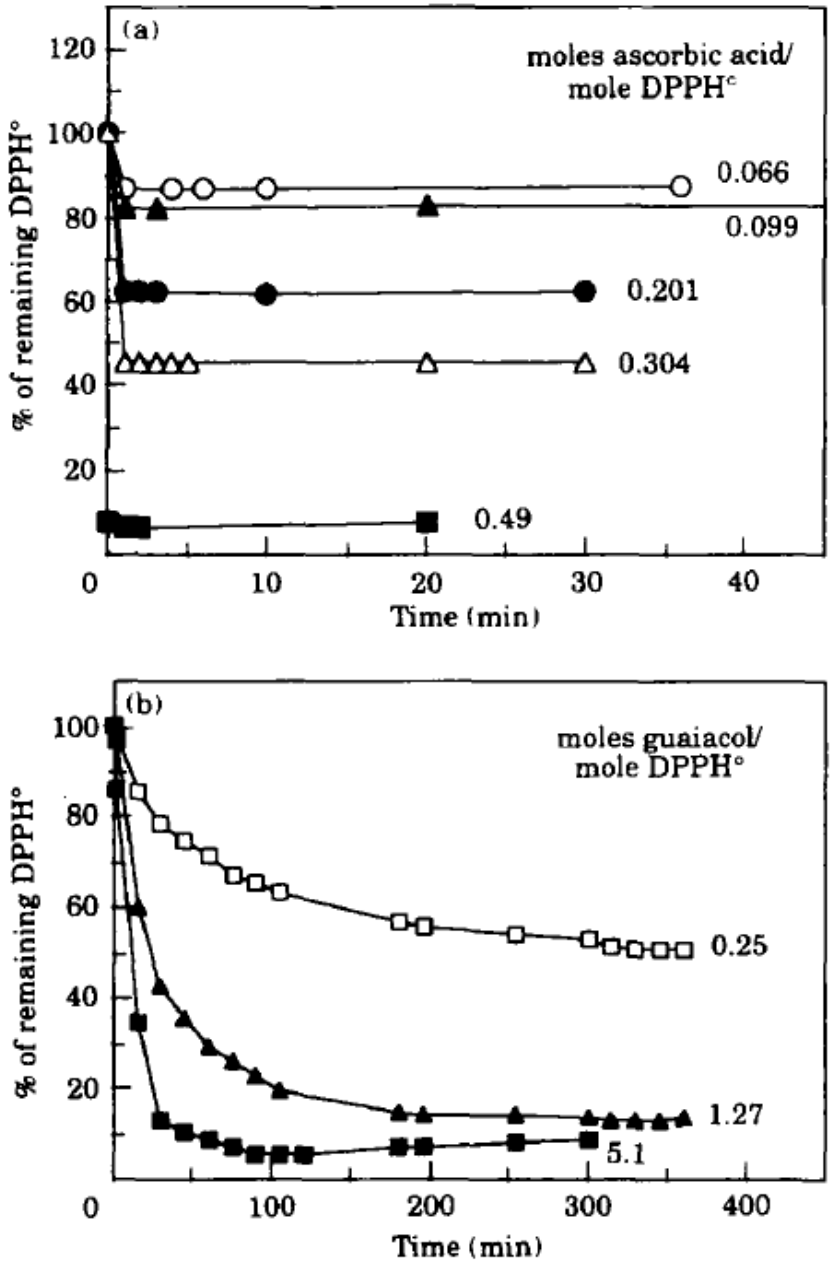


Fig. 1 Examples of the two observed types of reaction kinetics. (**a**) Kinetic behaviour of ascorbic acid; (**b**) kinetic behaviour of guaiacol

https://doi.org/10.1016/S0023-6438(95)80008-5

Expression the result

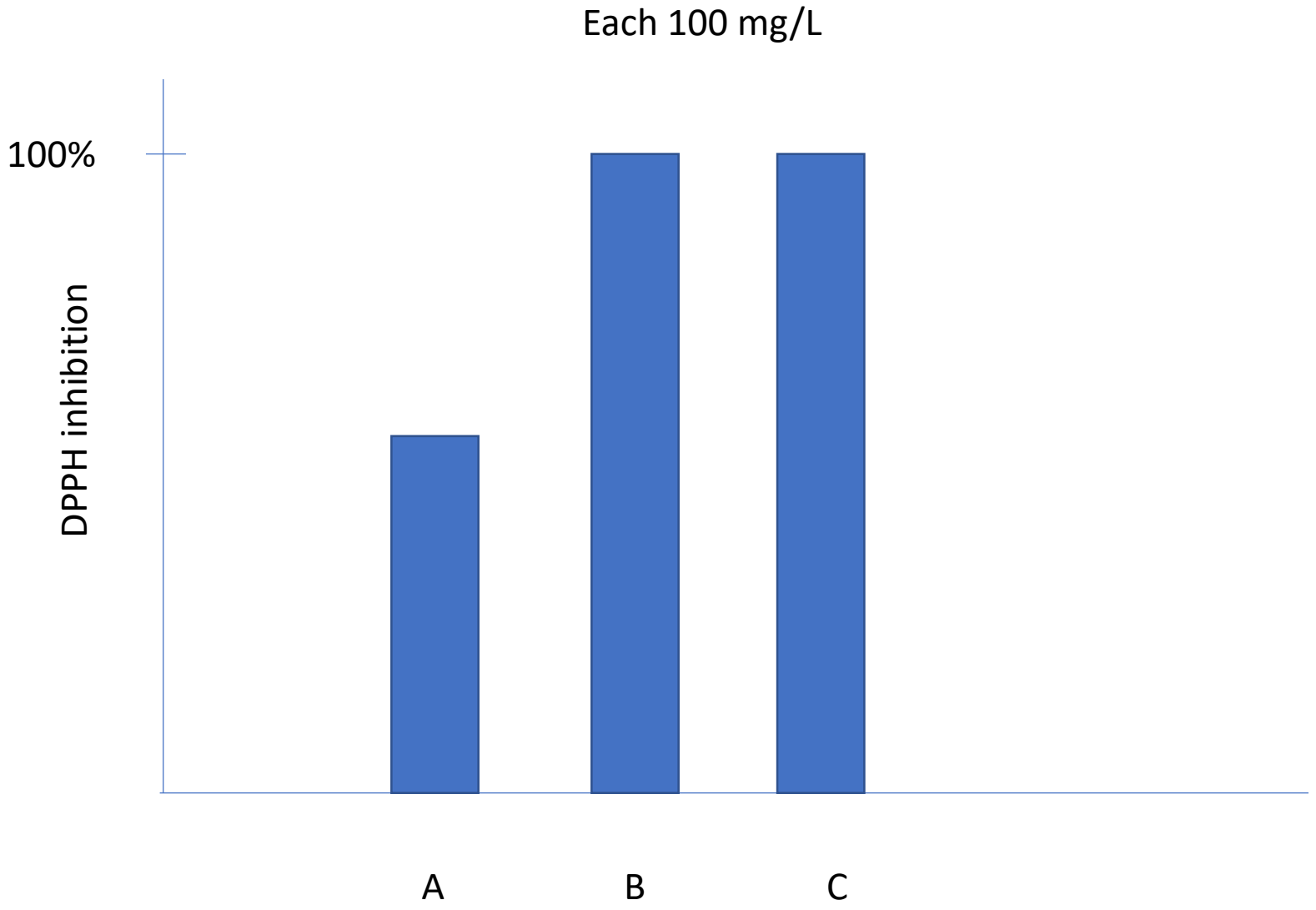
Scavenging capacity

$$\% \text{ inhibition} = \frac{A0 - A1}{A0} \times 100$$

A0 = absorbance of DPPH+blank
A1 = absorbance of DPPH+ sample

Caution:

- The value depends on the concentration of DPPH solution and of the sample used
- When the value near 100%, it's possible that too high concentration of the sample is used.



Which sample is the best antioxidant?

EC50 = amount of antioxidant necessary to decrease the initial DPPH concentration by 50%

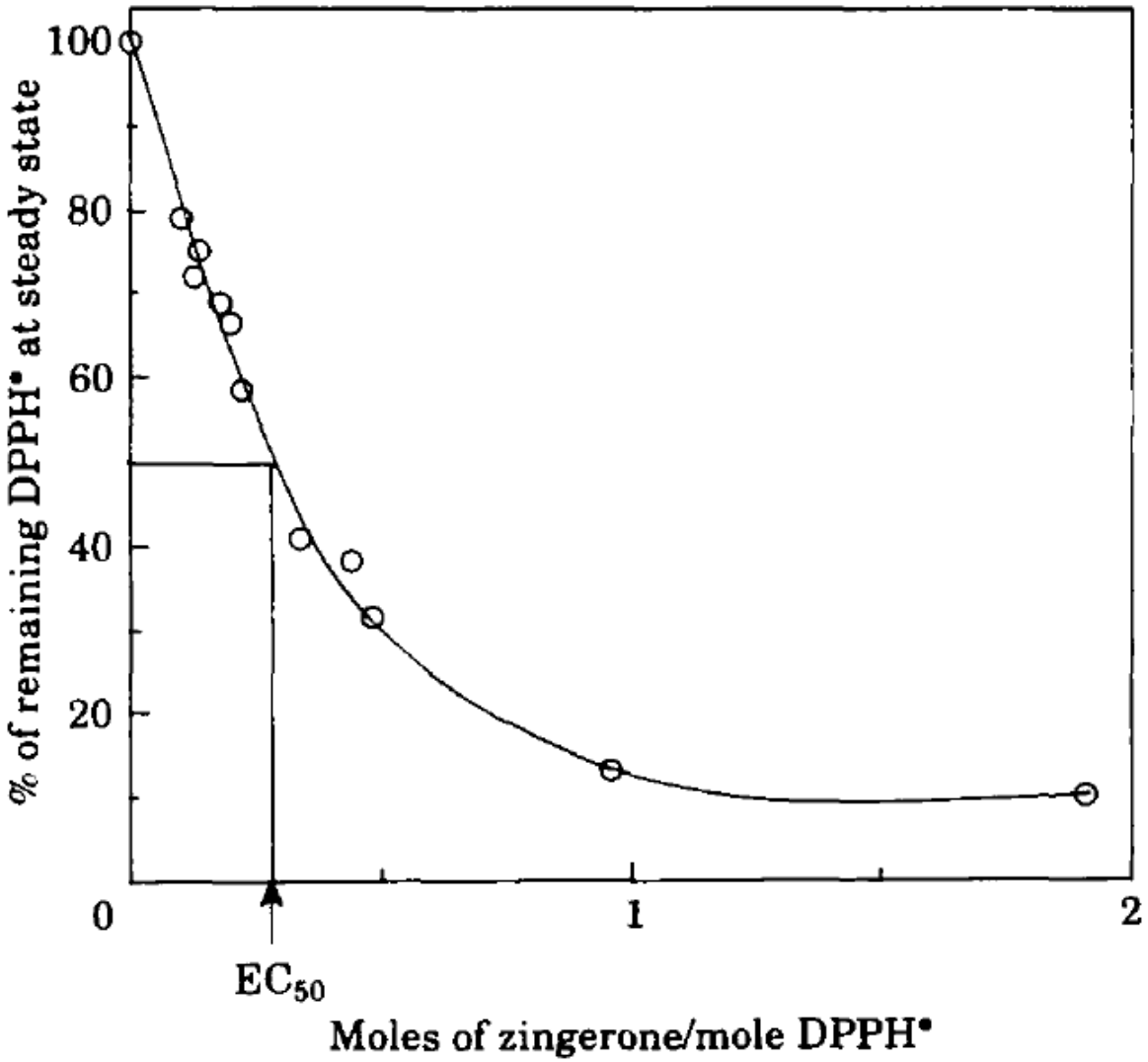


Fig. 2 The disappearance of DPPH• as a function of the number of moles of zingerone/mole DPPH•

https://doi.org/10.1016/S0023-6438(95)80008-5

Thank you