Binary HPLC method fot determination of 4-hydroxybenzoic acid (4-HBA), chlorogenic acid (CGA), quercetin (QUE), and salicilic acid (SAL)

Jun 29, 2021

Mariusz Krzysztof Janczur[1], Emilio González Camarena[1]

[1]Universidad Autónoma del Estado de México

1 Works for me Share

dx.doi.org/10.17504/protocols.io.mhac32e

Biolevolmex

Mariusz Krzysztof Janczur
Universidad Autónoma del Estado de México

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

ABSTRACT

We cut 1 g of each sample in such way that it contained the cuticle from one side of the sample. We homogenized it with 35 ml of the methanol 100%, and left them in ultrasonic 6 l bath during 30 min at room temperature. Methanol extracts obtained were filtered, placed in amber bottles to avoid degradation by light, and stored at –20°C until processed. We used Waters 717 liquid chromatograph with autosampler, Waters 2487 HPLC Absorbance UV-Vis Detector, Waters 1525 Binary HPLC Pump, Waters control module with SAT/IN Bus (Waters, Milford, MA, USA), Symmetry HPLC C18 column (particle size 5 µm, length 250 mm, internal Ø = 4.6 cm; Waters, Milford, MA, USA). We filtered the extracts with 0.45 µm pore size nylon-membrane filter. To obtain the calibration curves we used standards for salicylic acid (SAL), 4-hydroxybenzoic acid (4-HBA), chlorogenic acid (CGA), and quercetin (QUE), all supplied by Sigma-Aldrich. The mobile phase consisted of 0.1% v/v acetic acid (A) together with 100% acetonitrile (B). For the mobile phase A, we dissolved 1ml of glacial acetic acid with HPLC water, until 1 L. For the mobile phase B we used 100% acetonitrile. We filtered both mobile phases with 0.45 µm nylon membrane. We degasified them with ultrasonic bath during 30 min. We put the column temperature at 25°C, used the 254 nm UV detector, established the flow of the mobile phase at 0.2–0.8 mL/min, injection volume at 8 µL, and run time at 35 min. To wash the piston seals we used MeOH : H_2O (60 : 50). To quantify the four compounds, we used the calibration curves prepared out of 10 mg stock solution in 5 mL of MeOH. We took known concentrations from these solutions to obtain the relationship between the area corresponding to absorbance and concentration of each metabolite.

DOI

dx.doi.org/10.17504/protocols.io.mhac32e

PROTOCOL CITATION

Mariusz Krzysztof Janczur, Emilio González Camarena 2021. Binary HPLC method fot determination of 4-hydroxybenzoic acid (4-HBA), chlorogenic acid (CGA), quercetin (QUE), and salicilic acid (SAL). **protocols.io** https://dx.doi.org/10.17504/protocols.io.mhac32e

Citation: Mariusz Krzysztof Janczur, Emilio GonzÃÂ¡lez Camarena (06/29/2021). Binary HPLC method fot determination of 4-hydroxybenzoic acid (4-HBA), chlorogenic acid (CGA), quercetin (QUE), and salicilic acid (SAL). https://dx.doi.org/10.17504/protocols.io.mhac32e

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

KEYWORDS

4-hydroxybenzoic acid, 4-HBA, chlorogenic acid, CGA, quercetin, QUE

LICENSE

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jan 08, 2018

LAST MODIFIED

Jun 29, 2021

PROTOCOL INTEGER ID

9474

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Extraction of secondary metabolites

1 We cut 1 g of each sample in such way that it contained the cuticle from one side of the sample. We homogenized it with 35 ml of the methanol 100%, and left them in ultrasonic 6 l bath during 30 min at room temperature.

35 mL methanol

[M]100 % volume methanol

00:30:00

Mobile phase composition

2 The mobile phase consisted of 0.1% v/v acetic acid (A) together with 100% acetonitrile (B).
For the mobile phase A, we dissolved 1ml of glacial acetic acid with HPLC water, until 1 L. For the mobile phase B we used 100% acetonitrile.

Filtration of mobile phases

3 We used 0.45 µm nylon membrane.

Degasification of mobile phases

4 We degasified them with ultrasonic bath during 30 min.

Column temperature

Citation: Mariusz Krzysztof Janczur, Emilio GonzÃÂ¡lez Camarena (06/29/2021). Binary HPLC method fot determination of 4-hydroxybenzoic acid (4-HBA), chlorogenic acid (CGA), quercetin (QUE), and salicilic acid (SAL). https://dx.doi.org/10.17504/protocols.io.mhac32e

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

5 We put the column temperature at 25°C

UV detection light frequency

6 We used the 254 nm UV detector

Mobile phaase flow rate

7

Time	Flow rate (ml/min)	%A (acetic acid)	%B (acetonitrile)
0.00	0.30	80.0	20.0
5.30	0.30	70.0	30.0
11.00	0.20	65.0	35.0
13.00	0.20	50.0	50.0
13.30	0.20	40.0	60.0
15.00	0.20	30.0	70.0
16.00	0.30	40.0	60.0
17.00	0.70	50.0	50.0
19.00	0.70	65.0	35.0
22.0	0.80	70.0	30.0
30.0	0.80	80.0	20.0
35.0	0.80	80.0	20.0

Calibration curves

8 To quantify the four compounds, we used the calibration curves prepared out of 10 mg stock solution in 5 mL of MeOH. We took known concentrations from these solutions to obtain the relationship between the area corresponding to absorbance and concentration of each metabolite. We obtained the fallowing calibration curves:
$y_i = 1109.4x_i + 481.67$,
$y_i = 296.01x_i + 133.74$,
$y_i = 551.41x_i + 263.64$, and
$y_i = 919.96x_i + 201.64$,
where y_i – area below the absorbance curve, x_i – concentration of the secondary metabolite, and $i = 1, 2, 3$ or 4 for 4-hydroxybenzoic acid (4-HBA), chlorogenic acid (CGA), quercetine (QUE), or salicilic acid (SAL), respectively.

9

Citation: Mariusz Krzysztof Janczur, Emilio GonzÃÂ¡lez Camarena (06/29/2021). Binary HPLC method fot determination of 4-hydroxybenzoic acid (4-HBA), chlorogenic acid (CGA), quercetin (QUE), and salicilic acid (SAL). https://dx.doi.org/10.17504/protocols.io.mhac32e

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited