DEVELOPMENT OF AN LC/MS/MS METHOD TO SEPARATE AND ANALYZE CURCUMINOIDS, THEIR METABOLITES AND DEGRADATION PRODUCTS

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ABSTRACT

Turmeric has been used in Ayurvedic medicine for thousands of years. One recent report by Keun et al. [1] has also been made in a coloring agent and as a spice in many fields. The curcumin, mainly curcumin, demethoxycurcumin, and bisdemethoxycurcumin, are the principal compounds responsible for the yellow color of turmeric. The biological activity of curcumin, as the major component of turmeric, has also been studied in recent years. Its anti-inflammatory and antioxidant properties make it an attractive therapeutic agent for the treatment of various diseases. In addition, curcumin has been studied in preclinical models for the promotion of degradation products might interfere with the analytical detection. The main goal of this study was to develop an LC/MS/MS method to satisfactorily and accurately determine the performance of curcuminoids and to establish a bioanalytical method for the separation of curcuminoids, their metabolites and degradation products. The separation of the major curcuminoids was achieved using a single analytical method for every compound.

EXPERIMENTAL PROCEDURE

- A reversed-phase gradient HPLC and LC/MS/MS method were developed to separate and identify curcuminoids (C), demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), vanillin, vanillic acid and ferulic acid in a single analytical method.
- Agilent 1100 HPLC system (quaternary gradient pump, degasser, diode array detector, thermostat-controlled column compartment and autosampler) with a Zebron ZB-C18 column (5 µm, 150 x 4.6 mm), and C18 guard cartridge (4.0 x 3.0 mm) was used to obtain the HPLC chromatograms of 386 µg/mL C (8) standards in acetonitrile. Mobile phase: A = 0.05% acetic acid in Nanopure water; B = 0.05% acetic acid in acetonitrile; C = 1% ammonium acetate; D = 1% ammonium acetate in acetonitrile.
- The LC/MS system used was Agilent N600 Triple Quad, for a total mass spectrometer in negative electrospray ionization (ESI) mode was used to obtain MS/MS spectra. The parameters for each compound were optimized performing optimization by infusing the compounds separately. The negative ESI mass spectrometer method was used to its optimal settings for every compound.
- Mass spectrometric data were acquired in a flow set at 5.0 ml/min. The drying gas pressure was set at 60 psi. The drying gas was provided at 350°C, and capillary voltage was 4000 V.
- Curcumin, demethoxycurcumin, and bisdemethoxycurcumin analytical standards were obtained and purified from turmeric rhizome. Curcuminoids were provided by Dr. M. H. Demir (The University of Sheffield, UK). Vanillin and vanillic acid were obtained from Fluka, Buchs, Switzerland. All standards were prepared in acetonitrile.

CONCLUSIONS

- Satisfactory HPLC separation was achieved for all the tested compounds by changing the composition of the mobile phase.
- The mass spectrometric data for all the compounds were acquired in a flow set at 5.0 ml/min. The drying gas pressure was set at 60 psi. The drying gas was provided at 350°C, and capillary voltage was 4000 V.
- Curcumin, demethoxycurcumin, and bisdemethoxycurcumin analytical standards were obtained and purified from turmeric rhizome. Curcuminoids were provided by Dr. M. H. Demir (The University of Sheffield, UK). Vanillin and vanillic acid were obtained from Fluka, Buchs, Switzerland. All standards were prepared in acetonitrile.

REFERENCES


ACKNOWLEDGMENTS

We thank Dr. Marko (IAFS) for providing curcuminoids and their metabolites and degradation products. We also thank Dr. M. H. Demir (University of Sheffield, UK) for providing curcuminoids and their metabolites. This work was supported by NIH Grant P01 CA58078.