Quantification of Naringinen, Bergamottin, and 6', 7'-Dihydroxybergamottin-Three Major Components in Grapefruit Juice by LC-MS/MS

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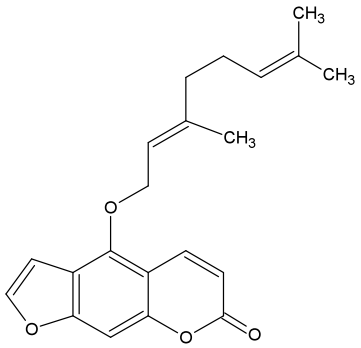
Introduction

Components of grapefruit juice irreversibly inhibit drug-metabolizing enzymes such as CYP3A4 and can alter the pharmacokinetic and pharmacodynamic parameters of the drugs. A number of studies have been reported for the investigation of drug-grapefruit juice interactions. However, very few studies were devoted to the analysis of the components in grapefruit juice. This poster presents a high throughput LC-MS/MS method for the simultaneous determination of naringinen, bergamottin, and 6',7'-dihydroxybergamottin in grapefruit juice for support of a drug-grapefruit juice interaction study.

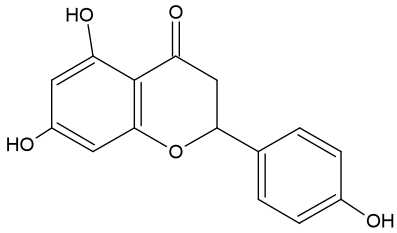
Methods and Instrumentation

Sample Preparation: Naringinen, bergamottin, 6', 7'-dihydroxybergamottin, and psora-4, and apigenin A as the internal standards were purchased from Sigma-Aldrich (Figure 1). A simple dilution procedure was used for sample preparation. All samples, quality control samples, and standards with a sample volume of 0.05 mL spiked with IS, were diluted with 1 mL of methanol (MeOH). After centrifuge at 3500 rpm for 5 minutes, 0.1 mL of supernatant was transferred into sample vial with 0.9 mL of 1:1 MeOH/H_2O (v/v) with 0.2 % formic acid which was injected for LC-MS/MS analysis after mixing. All the calibration standards and QC samples were prepared in purified water except for QC-Mid-2 which was prepared from grapefruit juice and its concentration was determined by spiking analytes into blank grapefruit juice with a background level determined from the mean of 6 injections.

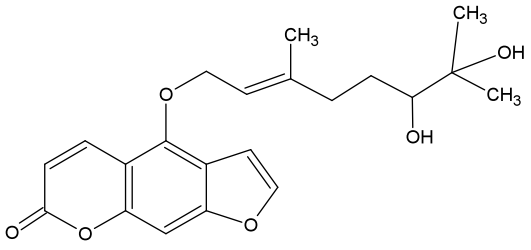
Figure 1. Chemical Structures of Naringinen, Bergamottin, and 6',7'-Dihydroxybergamottin



Bergamottin



Naringenin



6 ,7 -Dihydroxybergamottin

HPLC Conditions: Chromatographic separation was achieved on a Gemini C6-Phenyl column (50 x 3 mm, 3 micron) under gradient condition at a flow rate of 0.4 mL/min. Mobile phase A was 0.1% formic acid in water. Mobile phase B was 0.1% formic acid in MeOH. Total run time was 5 minutes. Shimadzu LC pumps and autosampler were used for solvent delivery and sample injection.

Mass Spectrometric Conditions: MS/MS detection was performed in turbo ionspray positive mode with a Sciex API 5000 MS/MS System. The mass transitions (precursor to daughter, m/z) monitored were m/z 339.1→203.0 for bergamottin, m/z 273.2→203.0 for 6',7'-dihydroxybergamottin and m/z 273.1→152.9 for naringinen, respectively, using psora-4 (335.0→90.9 m/z) as the internal standard for bergamottin and apigenin (271.1→152.9) as the internal standard for naringinen and 6',7'-dihydroxybergamottin.

Results

The developed LC-MS/MS method was fully validated for precision, accuracy, sensitivity, stability, and calibration range. There was no interference between the compounds - naringinen, bergamottin, and 6', 7'-dihydroxybergamottin. The linearity of the method was evaluated at a linear range of 0.1 µg/mL-20 µg/mL for all three analytes. The results of LLOQ samples are shown in Table 1. All calibration curves have a coefficient of determination (r^2) ≥ 0.9976. The intra-run and inter-run precision and accuracy for naringinen, bergamottin, and 6',7'-dihydroxybergamottin are summarized in Table 2, which demonstrated that there is no matrix difference between purified water and grapefruit juice.

Table 1. Results of LLOQ (0.1 µg/mL) samples (N=6)

Bergamottin			6',7'-Dihydroxybergamottin			Naringenin		
Mean	%CV	%Nominal	Mean	%CV	%Nominal	Mean	%CV	%Nominal
0.101	5	101	0.097	8.2	97	0.103	2.9	103

Table 2. Precision and accuracy of QC samples

Intra-assay (N=6)

Nominal	Bergamottin			6',7'-Dihydroxybergamottin			Naringenin		
(µg/mL)	Mean	%CV	%Nominal	Mean	%CV	%Nominal	Mean	%CV	%Nominal
0.3	0.293	2.7	97.7	0.292	4.5	97.3	0.301	2.7	100.3
4	3.975	3.9	99.4	3.982	2.5	99.6	3.987	1.1	99.9
5.64/5.55/3.99	5.639	3.8	100.0	5.254	3.5	94.7	3.95	2.7	98.8
15	14.338	4.4	95.6	14.645	3.6	97.6	14.623	1.5	97.5

Inter-assay (N=18)

Nominal	Bergamottin			6',7'-Dihydroxybergamottin			Naringenin		
(µg/mL)	Mean	%CV	%Nominal	Mean	%CV	%Nominal	Mean	%CV	%Nominal
0.3	0.297	3.0	99.0	0.292	4.5	97.3	0.3	3.7	100
4	3.984	3.0	99.6	3.854	3.3	99.4	3.943	2.4	98.8
5.64/5.55/3.99	5.894	7.2	104.5	5.305	5.5	95.6	3.998	4.7	100.0
15	14.519	3.5	96.8	14.406	3.1	96.0	14.483	1.9	96.6

Conclusions

A simple, rapid, and reproducible LC-MS/MS method for the simultaneous determination of naringinen, bergamottin, and 6', 7'-dihydroxybergamottin in grapefruit juice has been developed and validated. The validated method has excellent precision and accuracy and has been successfully used for support of the drug-grapefruit juice interaction studies.