Use of an Unconventional Approach to Boost Sensitivity and Impr Chromatography for Quantifying Curcumin and Tetrahydrocurcumin MS/MS

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Introduction

Curcumin possesses multiple attractive pharmacological activities and it is be actively developed to treat several serious diseases, e.g. cancer and neurolo disorders. To support drug development programs, sensitive LC-MS methods curcumin and its metabolite, tetrahydrocurcumin in biological matrices, are essential. However, due to instability, all existing methods have used acidic r phases, which led to low sensitivity in the commonly used negative ionizatio poor chromatography, and split peaks. This is especially problematic for tetrahydrocurcumin because its concentration in various matrices is quite lo is most subject to these drawbacks. Contrary to conventional thinking, basic phases were evaluated to significantly increase the detection sensitivity and same time improve the chromatography for curcumin and tetrahydrocurcumi

Methods

The analytes and their stable-isotope labelled internal standards were extrac from acidified human EDTA plasma or other matrices by supported liquid ext or liquid-liquid extraction with MTBE. The residues from sample extraction w reconstituted in acetonitrile/H_2O (60/40, v/v) and chromatographic separatio achieved on a Durashell C18 column (4.6 x 50 mm, 5 µm) with a basic mobil phase, acetonitrile/H_2O (60/40, v/v) + 0.2% NH_4OH. The MS detection was i negative mode using the mass transitions of 367.1→134.0, 371.2→235.1, 373.1→151.9, and 377.2→237.9 for curcumin, tetrahydrocurcumin, and thei internal standards, respectively. Curcumin and tetrahydrocurcumin were elut 0.7 min and 1.1 min, respectively (2 min run time). Quantification was base quadratic calibration with the weighting factor of $1/x^2$.

Preliminary Data

In comparison with the commonly used acidic mobile phases, curcumin and tetrahydrocurcumin were eluted as single symmetric chromatographic peaks the basic separation conditions. Because of the elimination of multiple peaks due to already negatively charged analytes in the basic mobile phase, the de

sensitivity in negative mode on mass spectrometer increased by more than 4
13-fold for curcumin and tetrahydrocurcumin, respectively. Although the ana
are not very stable in basic conditions for an extended period of time, the ve
residence time in the LC column (both analytes completed eluted within 1.2 r
under basic conditions did not result in significant degradation of the analyte
the reconstitution solution was neutral, sufficient autosampler stability durati
(more than 72 hrs) was still obtained.

Slightly upward quadratic responses were consistently observed for both ana
over the concentration range of 5-5000 ng/ml. Hence, quadratic regression w
used for the regression. Satisfactory accuracy and precision were obtained. T
mean biases for QCs were all within ±5% and the coefficients of variation (C
were within 5%, except the CV of low QC for curcumin, which was 8.8%. The
no significant matrix effect across different human plasma lots. The CVs of in
standard normalized matrix factors were 3.2% and 1.4% for curcumin and
tetrahydrocurcumin, respectively.

Though more validation tests are still in progress, these preliminary results a
demonstrate the feasibility of this unconventional approach resulting in a sig
increase in the sensitivity and improvement of chromatography for curcumin
tetrahydrocurcumin. Based on these successful results, it is predicted that th
unconventional approach may be generally applicable to many other similar
challenging chromatographic situations.

Novel Aspect

First successful effort to use basic mobile phase to significantly increase sens
for potentially unstable analytes (curcumin and tetrahydrocurcumin)