# **Development of an LCMS Method to Detect and Quantify Curcumin in a Novel Oral Formulation of Turmeric**

## BACKGROUND

- Curcumin is a chemical produced from plants that belong to the Curcuma longa species. It is the main secondary metabolite of turmeric which can be classified under the ginger family(1).
- Curcumin was originally used to treat abdominal pains, sprains, and swelling. More current research has directed its use towards reduction of inflammation(2).
- ProCaps Laboratories has developed a new formulation of Turmeric, but the oral absorption is not known in humans. To answer this question the formulation should be tested in a Caco-2 model of in-vitro oral absorption followed by a clinical trial in humans. Prior to this work, however, an analytical method is needed to detect Curcumin. Liquid chromatography mass spectrometry (LCMS) is a highly sensitive and selective analytical method that can be used for this purpose.

### PURPOSE

• The objective of this study is to find an analytical method to detect Curcumin through Liquid Chromatography Mass Spectrometry (LCMS).

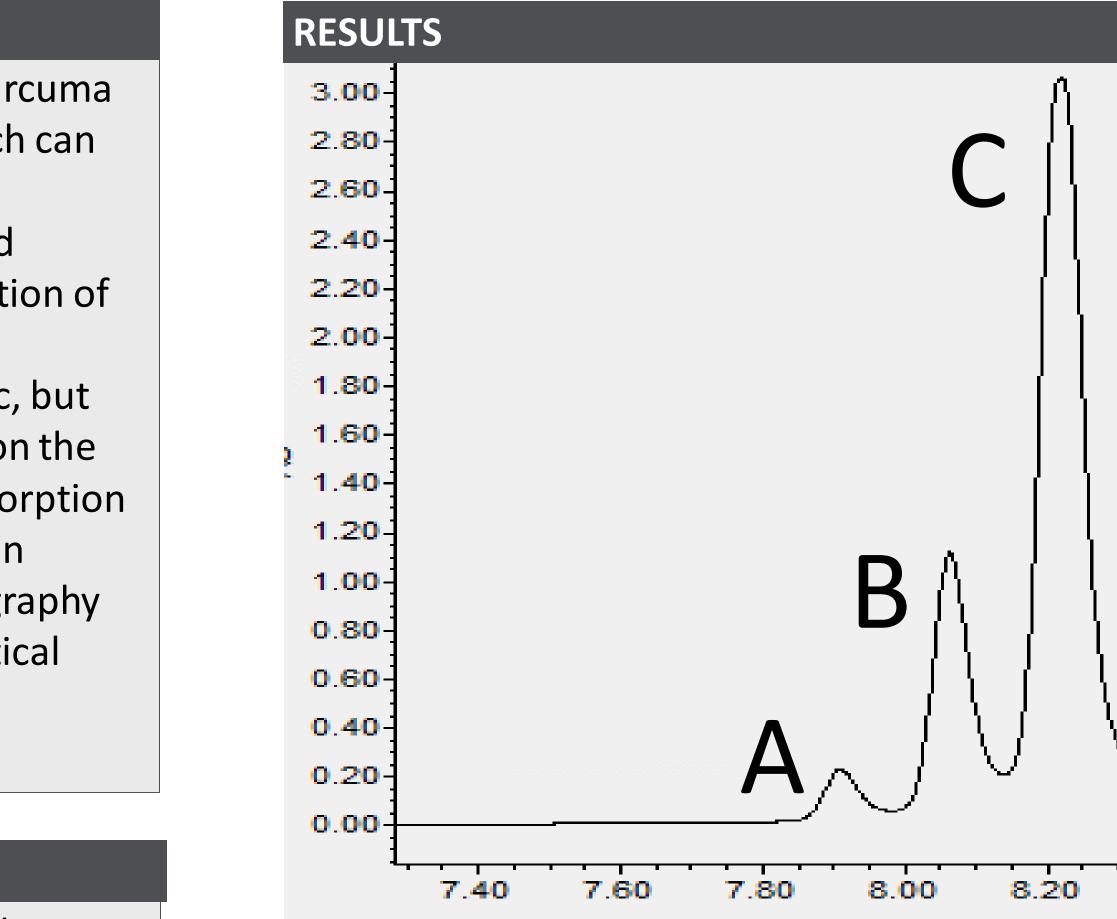
#### METHODS

- Using a group of high-performance liquid chromatography (HPLC) methods from USP, Chromadex, and ProCaps, a curcuminoid standard containing curcumin and two of its metabolites (bisdemethoxycurcumin and demethoxycurcumin) was run on a Waters Alliance HPLC system (Model 2965) with a photodiode array detector (Model 2998), and through a Waters Acquity QDa Mass Spectrometer.
- Empower3 software was used for sample analysis.
- The Curcuminoid peaks elute very close to each other, making clear differentiation of each UV peak difficult.
- Mass detection was incorporated into a new experimental method to monitor individual compounds and improve baseline resolution.
- The experimental HPLC method used a 95:5 H20:Acetonitrile gradient while setting UV detection at 425 nm as seen in Table 1.
- MS Scan and single ion recording (SIR) parameters are listed in Table 2.
- The M/Z ratio of Curcumin was identified by finding the strongest signal in the mass chromatogram.

| <b>CHEMICALS</b> | TRUCTURE OF CURCUMINOIDS |                         |
|------------------|--------------------------|-------------------------|
| Curcumin         | Demethoxycurcum          | in Bisdemethoxycurcumin |
|                  | ()                       | Сон<br>HO               |

## Brandon Renninger<sup>1,2</sup>, Amritpal Badwalz<sup>2</sup>, Jeffery Dorsett<sup>3</sup>, Mohammad Khalid<sup>3</sup>, Casey L. Sayre<sup>1</sup>

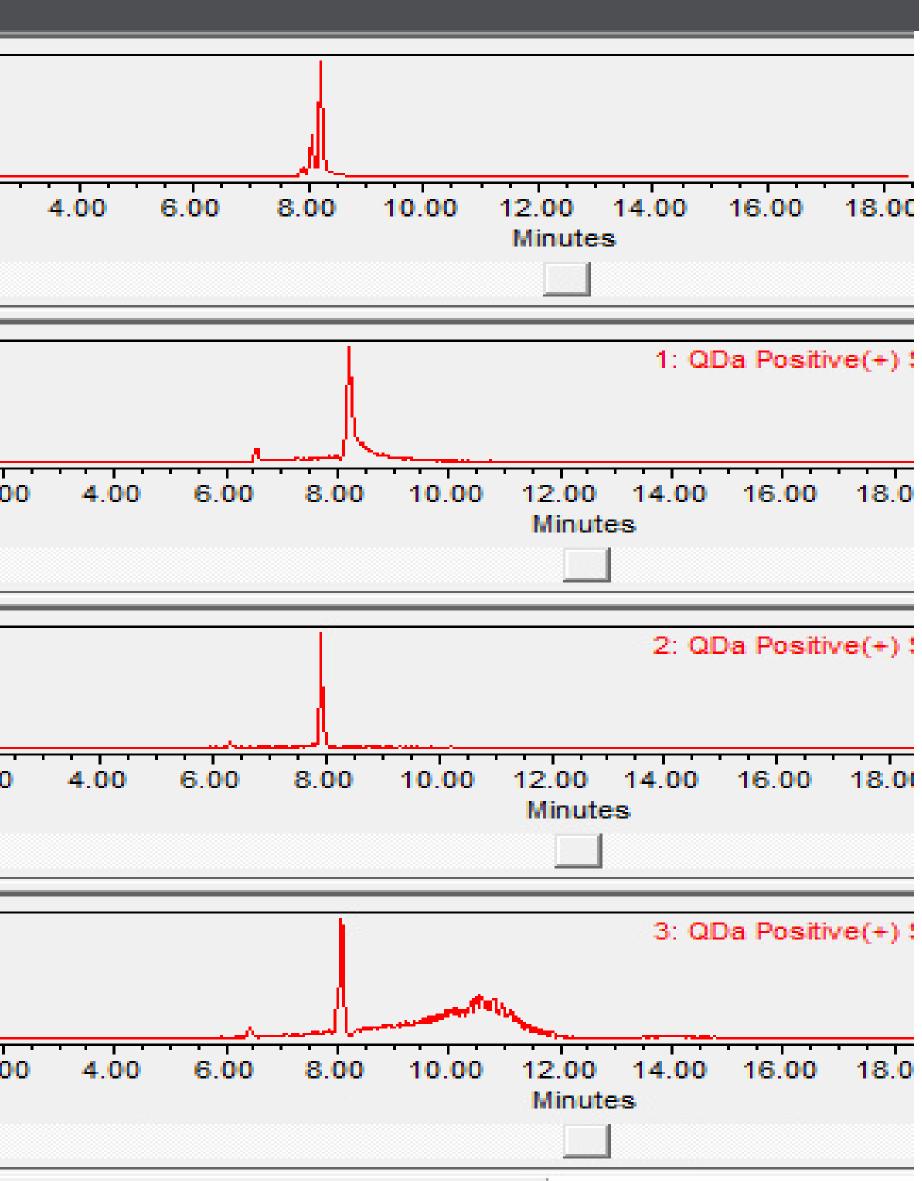
<sup>1</sup> Roseman University of Health Sciences, College of Pharmacy, South Jordan, UT <sup>2</sup>Roseman University of Health Sciences, College of Graduate Studies, South Jordan, UT <sup>3</sup>ProCaps Laboratories, Henderson, NV



| RESULTS  |   |                                |           |               |    |                                  | RESULTS  |  |
|--|---|--------------------------------|-----------|---------------|----|----------------------------------|--|--|
| 3.00<br>2.80<br>2.60<br>2.40<br>2.20<br>2.00<br>1.80<br>1.60<br>1.40<br>1.20<br>1.00<br>0.80<br>0.60<br>0.40<br>0.20<br>0.00 | A   |                                |           |               |    |                                  |  |  |
| Peak A. Bisdeme  | 7.60 7.80<br>JV PDA detection<br>ethoxycurcumin;<br>Fig 1 is a zoomed<br>Flow(ml/ | Peak B. Deme<br>l in version o | ethoxycur | cumin; Peak C | 2. | 9.00<br>(ACN)                    | III.<br>100000-<br>0.00 2.00<br>IV.                        |  |
| 0  | 1.00  |                                | 95        |               | 5  |                                  | Figure 2. UV/MS  |  |
| 10.99  | 1.00  |                                | 5         |               | 95 |                                  | I. UV chromate<br>II. MS SIR of Cu<br>III. MS SIR of Big   |  |
| 15.99  | 1.00  |                                | 95        |               | 5  |                                  | IV. MS SIR of De   |  |
| Table 1. Gradient f  | flow for HPLC   |                                |           |               |    |                                  | CONCLUSIONS  |  |
| Method   | Polarity  | Start(m                        | in)       | Stop(min)     |    | Function<br>Details              | In conclusion, ea<br>to each other. He                     |  |
| MS Scan  | Positive  | 0.00                           |           | 25.00         |    | Scan Mass<br>200 Da to 500<br>Da | of Mass Spectros<br>detected accurat                       |  |
| SIR  | Positive  | 0.00                           |           | 25.00         |    | SIR of mass<br>369.38 Da         | 1. Hewlings SJ, Ka<br>Human Health. Fo<br>2. Anand P, Thor |  |
| SIR  | Positive  | 0.00                           |           | 25.00         |    | SIR of mass<br>339.35 Da         | Harikumar KB, Su<br>analogues (Conge<br>Pharmacol. 2008;   |  |
| SIR  | Positive  | 0.00                           |           | 25.00         |    | SIR of mass 309.33               | ROSEN<br>O F H E   |  |
| Table 2 ODA MAS D  | aramatara   |                                |           |               |    |                                  |  |  |

| RESULTS  |   |                 |                                  | RESULTS  |
|--|---|-----------------|----------------------------------|--|
| 3.00-<br>2.80-<br>2.60-<br>2.40-<br>2.20-<br>1.80-<br>1.60-<br>1.40-<br>1.20-<br>1.00-<br>0.80-<br>0.60-<br>0.40-  |   |                 |                                  |  |
| 0.20<br>0.00<br>7.40<br>7.60<br>7.80<br>8.<br>Figure 1. HPLC UV PDA detection of<br>Peak A. Bisdemethoxycurcumin; Pe<br>Curcumin. Note Fig 1 is a zoomed i | eak B. Demethoxycu<br>n version of Fig 2 I. | rcumin; Peak C. |                                  |  |
| Fime(min) Flow(ml/m  | nin) %A(H2                                  | 0)              | %B(ACN)                          |  |
| 1.00   10.99 1.00  | 95<br>5                                     |                 | 5<br>95                          | Figure 2. UV/MS<br>I. UV chromate<br>II. MS SIR of Cu      |
| 15.99 1.00   | 95  | Ę               | 5                                | III. MS SIR of Bis<br>IV. MS SIR of De                     |
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| AS Scan Positive   | 0.00  | 25.00           | Scan Mass<br>200 Da to 500<br>Da | of Mass Spectros<br>detected accurat                       |
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- S data overlay in real time.
- atogram of all three Curcuminoids
- Curcumin
- Bisdemethoxycurcumin
- Demethoxycurcumin

each Curcuminoid compound eluted closely in time However, with the added specificity

oscopy, each individual compound peak can be rately with baseline resolution and separation.

Kalman DS. Curcumin: A Review of Its Effects on Foods. 2017;6(10).

omas SG, Kunnumakkara AB, Sundaram C,

Sung B, et al. Biological activities of curcumin and its geners) made by man and Mother Nature. Biochem 8;76(11):1590-611.

