

# Simultaneous Determination of Curcumin and Berberine in their Pure Form and from the Combined Extracts of *Curcuma Longa* and *Berberis Aristata*

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**Abstract:** A simple, rapid, accurate, precise, reliable and economical first derivative spectrophotometric method has been proposed for the simultaneous determination of curcumin (CUR) and berberine (BER) in pure form and in combined methanolic extracts of *Curcuma longa* and *Berberis aristata* without any prior separation or purification step. The developed methods show best results in terms of linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) for standard laboratory mixtures of pure compounds and methanolic extracts of the drugs. The linearity range for curcumin (CUR) and berberine (BER) were found to be 2 to 8  $\mu\text{g mL}^{-1}$  and 6 to 12  $\mu\text{g mL}^{-1}$  respectively. The values of LOD were 0.039  $\mu\text{g mL}^{-1}$  and 0.025  $\mu\text{g mL}^{-1}$  and the values of LOQ were found to be 0.13  $\mu\text{g mL}^{-1}$  and 0.08345  $\mu\text{g mL}^{-1}$  for curcumin (CUR) and berberine (BER) respectively. By this method, *C. longa* and *B. aristata* showed 4% w/w curcumin and 2.8% w/w berberine respectively in combined methanolic extracts of the two herbal drugs. The method was validated as per ICH guidelines.

**Keywords:** simultaneous determination; derivative spectroscopy; curcumin; berberine; *Curcuma longa*; *Berberis aristata*

## 1. Introduction

Curcumin (CUR, 1), 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadien-3, 5-dione, is a polyphenol derived from the herbal remedy and dietary spice turmeric (*Curcuma longa*). It possesses diverse anti-inflammatory and anti-cancer properties following oral or topical administration [1]. Turmeric and curcumin are reported to be potent anti bacterial and anti hepatotoxic agents [2]. Berberine is the major alkaloidal component of *Rhizoma coptidis*, bark of *Berberis aristata* and *Berberis lycium*. Berberine is a quaternary ammonium salt from the group of isoquinoline alkaloids with a bright yellow color that is easily seen

in most of the herb materials that contain any significant amount of this compound. It has multiple pharmacological effects including inhibiting acetylcholinesterase, reducing cholesterol and glucose, lowering mortality in patients with chronic congestive heart failure and anti-inflammation etc [3]. Berberine and *B. aristata* extract are known to be good anti – bacterial, anti-protozoal, anti- hepatotoxic and wound healing agents. In addition, berberine also used in the treatment of diabetes, hyperlipemia, coronary artery disease and ischemic stroke etc. [4]. Recent studies indicate that curcumin (CUR) and berberine

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(BER), given along with andrographolides, are effective in the treatment of malaria [5].

Some procedures have been described for the determination of either curcumin (CUR) or berberine (BER) from the source material [6-8]. HPLC, spectrophotometric and TLC methods have been reported for determination of curcumin (CUR) in their various plant species [9-10]. TLC, HPLC, spectroscopic, HPTLC, spectrofluorimetric methods have been reported for the determination of berberine in various plant species [11-15]. Simultaneous determination of berberine along with palmatine and jatrorrhizine by liquid chromatography–tandem mass spectrometry in rat plasma and its application in a pharmacokinetic study after oral administration of coptis–evodia herb couple has been reported [16]. There are polyherbal formulations incorporating both *C. longa* and *B. aristata*. Even though various analytical methods are available in literature for quantification of curcumin (CUR) and berberine (BER) from their source plants individually, no method is available for their simultaneous determination. Since both curcumin (CUR) and berberine (BER) have strong absorption in UV light, it was thought worthwhile to develop a spectrophotometric method for the simultaneous determination of both compounds from herbal extracts mixtures.

Spectrophotometric methods of analysis are more economic and simpler, compared to methods such as chromatography and electrophoresis. Under computer-controlled instrumentation, derivative spectrophotometry is playing a very important role in the multi-component analysis of mixtures by ultraviolet-visible molecular absorption spectrophotometry [17]. The aim of this work was to investigate the utility of derivative spectrophotometry and to develop reliable spectrophotometric procedures for the simultaneous determination of curcumin (CUR) and berberine (BER) in their pure and combined extract forms without any prior separation of individual drugs. Curcumin (CUR) and berberine

(BER) have closely overlapping spectra thus prevents the use of zero-order UV-Visible spectrophotometry for their determination. Derivative spectrophotometry is a very useful tool for overcoming this problem.

## 2. Materials and Methods

### 2.1. Instruments

Spectrophotometric measurements were made on a Shimadzu 1800 double beam UV Visible spectrophotometer with a fix slit width of 1 nm coupled with intex LCD computer loaded with Shimadzu UV PC software of version 2.3 and laser shot LBP-1210 Canon printer.

### 2.2. Reagents

All chemicals used were of analytical grade and double distilled water was used throughout. Pure CUR was obtained from Dishman pharmaceutical Pvt. Ltd., India and BER was obtained from Cadila Healthcare Pvt. Ltd., India. Methanolic Extracts of *C. longa* and *B. aristata* were prepared in the laboratory.

### 2.3. Solutions

Stock solutions, 0.1 mg mL<sup>-1</sup> in methanol, of pure samples of CUR and BER were freshly prepared individually in methanol. Stock solutions of extracts of *C. longa* and *B. aristata* were prepared in methanol.

### 2.4. Procedure

All reagents were tested for stability in solution and during the actual analysis. The behavior of the analytes remained unchanged up to about 72 h from their preparation when stored in refrigerator. CUR and BER were found to be stable during each kind of experimental measurements. Each measurement was done at room temperature.

#### 2.4.1. First derivative zero crossing method

The absorption spectra of pure drugs and their extract were recorded between 200 – 650 nm. In the first derivative method the scaling factor was kept as 1 and delta lambda was kept as 10. Calibration graphs were obtained by measuring the derivative ratio amplitudes against the increasing concentration of pure CUR and pure BER. For determination of CUR, in the presence of BER, 382 nm wavelength was selected where there is no interference of BER (Zero absorbance of BER). For determination of BER, in the presence of CUR, 259 nm wavelength was selected where there is no interference of CUR (Zero absorbance of CUR).

#### 2.4.2. Preparation for the extracts of *C. longa* and *B. aristata*

Rhizomes of *C. longa* and stem bark of *B. aristata* were procured from established traders in India and were authenticated in the pharmacognosy department of the institute. They were reduced to a fine powder of 40#. One gm of the above powder was extracted three times with 3 X 10 ml of methanol by refluxing for 1 h each time. The combined extracts of each drug were separately concentrated and final volume was adjusted to 25 ml with methanol for each drug separately.

### 2.5. Validation Parameters [18]

#### 2.5.1. Accuracy

Accuracy of proposed method and interference from extract was determined by recovery experiments. Recovery experiments were carried out by the standard addition method. This study was performed by addition of known amounts of CUR and BER to pre analyzed extracts. The amounts of standard recovered were calculated in terms of mean recovery with the upper and lower lim-

its of percent relative standard deviation.

#### 2.5.2. Precision

Intraday precision and inter day precision for the developed methods were measured in terms of % RSD. The experiments were repeated five times a day for intraday precision and on five different days for inter day precision. The concentration values for both intraday precision and interday precision were calculated five times separately and percent relative standard deviation were calculated. Finally the mean of % RSD ( $\% \text{RSD} = \frac{S}{X} \times 100$ , where S is standard deviation and X is mean of the sample analyzed) was calculated.

#### 2.5.3. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection (LOD) and Limit of Quantitation (LOQ) were calculated according to the 3 s/m and 10 s/m criterions, respectively, where s, is the standard deviation of the absorbance ( $n = 10$ ) of the sample and m is the slope of the corresponding calibration curve.

### 3. Results and Discussion

Curcumin and berberine obeyed Beer's law between the range of  $2 \mu\text{g mL}^{-1}$  to  $8 \mu\text{g mL}^{-1}$  and  $6 \mu\text{g mL}^{-1}$  to  $12 \mu\text{g mL}^{-1}$ , respectively. The absorption spectra of the two compounds, CUR and BER overlapped closely as shown in Figure 1.

For this reason, the determination of the above compounds was not possible by direct measurements of absorbance in zero-order spectra. On the other hand, derivative spectroscopy shows more resolution and makes it possible to analyze each drug in presence of one another as well as in the presence of other compounds of the extracts without any pre-treatment.

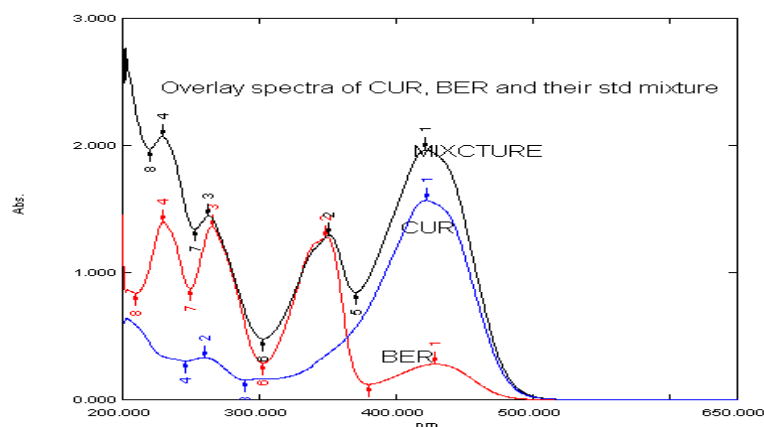


Figure 1. Zero order overlay spectra of CUR, BER and their standard mixture.

First derivative graphs of CUR and BER as well as overlay graphs of CUR and BER are shown in the Figure 2 to 4. Calibration graphs were obtained by measuring the derivative ratio amplitudes against the increasing concentration of pure CUR and pure BER. For determination of CUR, in the presence of BER, wavelength of 382 nm was selected where there is no interference of BER (Zero absorbance of BER) which is represented in Figure 5. For determination of BER, in the presence of CUR, wavelength of 259 nm was selected where there is no interference of CUR (Zero absorbance of CUR) as shown in Figure 6. The methanolic extracts of *C. longa*, when initially analyzed by zero order spectroscopy, showed  $4.1 \pm 0.269\%$  of curcumin. Similarly *B. aristata* extract showed  $2.81 \pm 0.524\%$  of berberine. Curcumin and berberine obey Beer's law between the range of  $2 \mu\text{g mL}^{-1}$ - $8 \mu\text{g mL}^{-1}$  and  $6 \mu\text{g mL}^{-1}$ - $12 \mu\text{g mL}^{-1}$  respectively. The two extracts of *C. longa* and *B. aristata* when mixed and analyzed for curcumin and berberine respectively by employing the proposed first order derivative spectroscopy, showed concentrations that closely matched with the results of zero order analysis of individual drugs which is represented in Table 1. The developed method was validated for accuracy and results of accuracy are shown in Table 2, summary of various validation parameters were listed in Table 3.

#### 4. Conclusion

The newly developed derivative spectrophotometric method for the simultaneous estimation of CUR and BER is simple, specific, accurate, precise, rapid and economical which indicates its adequacy for routine pharmaceutical analysis. It is concluded that derivative spectrophotometry is successfully utilized for the simultaneous estimation of CUR and BER in the pure combine forms as well as in combined extracts without any prior separation of individual drugs and in the absence of official monograph this method can be used for their determination. Based on the common therapeutic profile of *C. longa* and *B. aristata*, numbers of polyherbal formulations incorporating these two herbs are available in the market. The proposed method will be very useful for analyzing at least these two markers in such formulations. The proposed method is found to be simple, economical, rapid and at the same time reliable too.

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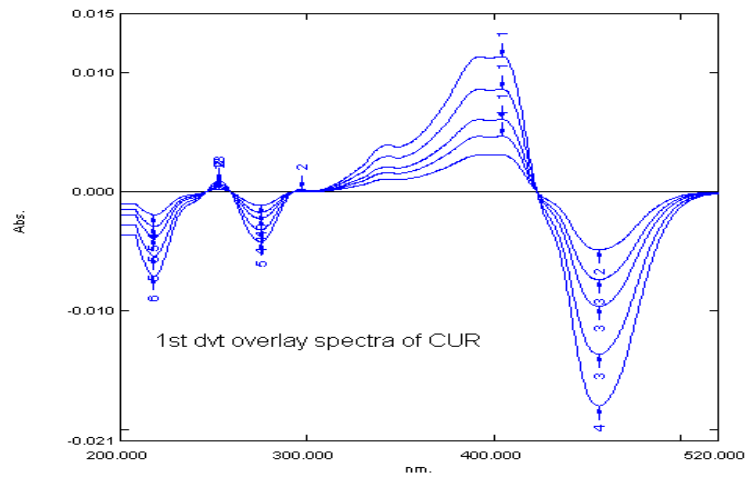


Figure 2. First derivative spectra of curcumin.

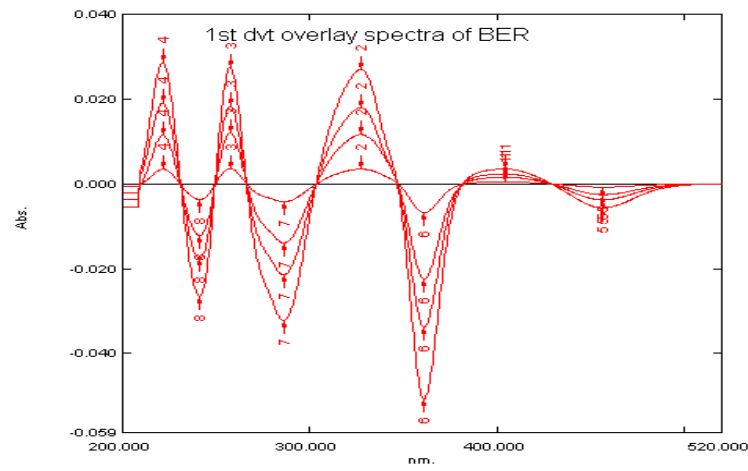


Figure 3. First derivative spectra of berberine.

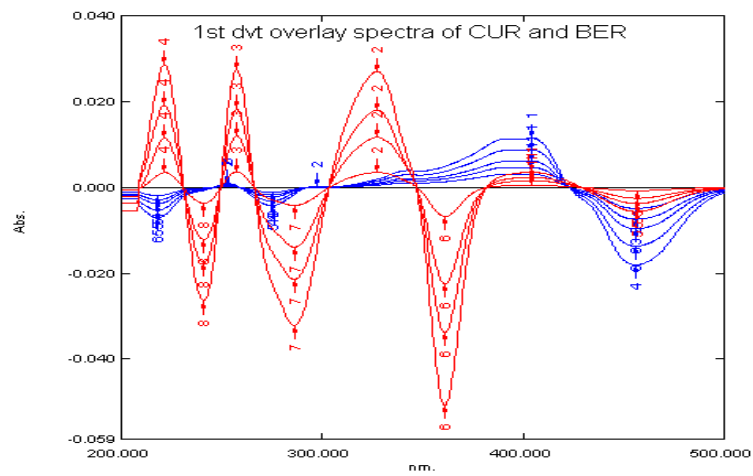


Figure 4. Overlain first derivative spectra of CUR and BER.

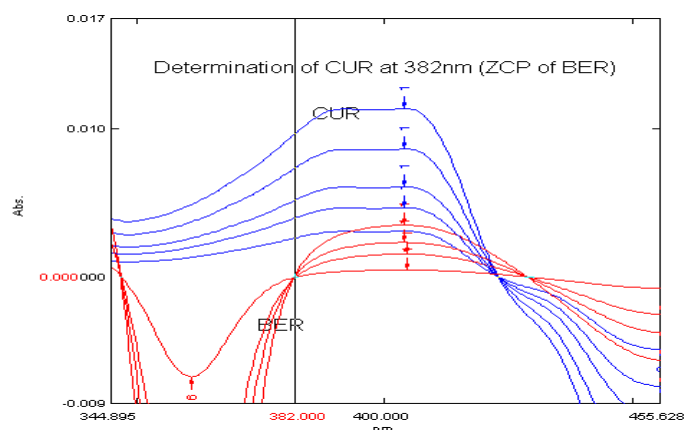


Figure 5. Determination of CUR at 382nm (zero crossing point of BER).

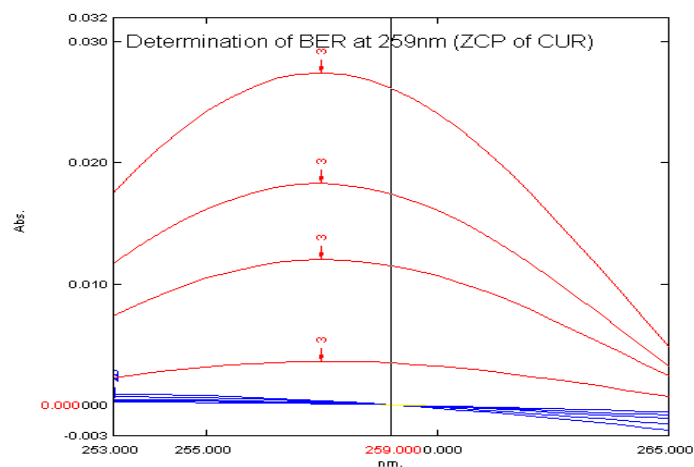


Figure 6. Determination of BER at 259nm (zero crossing point of CUR).

Table 1. Determination of curcumin and berberine from *C. longa* and *B. aristata* extracts.

Sr. no.	Sample	% w/w of marker compound	Method of determination
1	<i>C. longa</i> - methanolic extract (curcumin <sup>*</sup> )	4.1±0.269%	Zero order Uv – visible spectroscopy
2	<i>B. aristata</i> - methanolic extract (berberine <sup>**</sup> )	2.81±0.524%	Zero order Uv – visible spectroscopy
3	Extract of <i>C. longa</i> and <i>B. aristata</i> (mixture)	4.0±0.512% <sup>a</sup> 2.8±0.421% <sup>b</sup>	First derivative spectroscopy

\*Marker compound of sample of *C. longa*

\*\*Marker compound of sample of *B. aristata*

a Curcumin

b Berberine

Table 2. Results of recovery study of CUR and BER by the developed method.

Sr. no.	Amount of marker formed in the extracts		Amt of pure marker added ( $\mu\text{g mL}^{-1}$ )		% Recovery	
	CUR	BER	CUR	BER	CUR	BER
1	4.0 $\pm$ 0.512%	2.8 $\pm$ 0.421%	2.4	2.4	95.8	96.25
2			3	3	96.66	96.66
3			3.6	3.6	96.94	98.61
Mean					97.17	96.467
Standard Deviation					1.26	0.594

Table 3. Results of validation parameters obtained by the developed method

Validation parameters	Result obtained	
	CUR	BER
$\lambda_{\text{max}}$	382nm	259nm
Beer's law range ( $\mu\text{g mL}^{-1}$ )	2 $\mu\text{g mL}^{-1}$ -8 $\mu\text{g mL}^{-1}$	6 $\mu\text{g mL}^{-1}$ -12 $\mu\text{g mL}^{-1}$
Slop	0.001	0.0003
Intercept	0.000	-0.0017
Correlation coefficient	0.993	0.998
Accuracy	97.17 $\pm$ 1.26	96.467 $\pm$ 0.594
Precision (% RSD)	< 2 (% RSD)	< 2 (% RSD)
LOD ( $\mu\text{g mL}^{-1}$ )	0.039	0.025
LOQ ( $\mu\text{g mL}^{-1}$ )	0.13	0.08345

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