COMPARISON OF ANALYSIS METHODS FOR CURCUMIN DETERMINATION: A LITERATURE REVIEW

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ABSTRACT

Curcuminoids such as curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) are the major bioactive components found in Curcuma species, including turmeric (Curcuma longa L.). Curcumin is a polyphenol that is used as an antioxidant. Various analytical methods have been developed for the detection of curcumin, each with its advantages and limitations. Selection of an appropriate analytical method is crucial for obtaining accurate results. Although critical reviews on the chemical, biological, and pharmacological properties of curcumin are widely available, reviews specifically focusing on the different analytical methods for curcumin are limited. This review article aims to provide an overview of several analytical methods for the determination of curcumin. This is expected to encourage readers to choose a suitable analytical method for their specific needs. The articles used in this review were sourced from international English-language publications obtained from Science Direct, PubMed, and Google Scholar. The search was performed using keywords such as "Instrumental analysis of curcumin" and "Analytical techniques for curcumin analysis." The results reveal that spectrophotometric methods are simple techniques used to estimate curcumin content; however, their utility is limited to cases where the concentration of each curcuminoid is not a critical quality parameter. Accurate quantification and detection of trace amounts of curcuminoids and metabolites require the use of chromatographic separation methods combined with mass spectrometry detection (LC-MS/MS) because of their high accuracy, reproducibility, and sensitivity with low limits of detection (LOD) and lower limits of quantification (LLOQ).

Keywords: Analytical methods, Curcumin, Determination

INTRODUCTION

The primary bioactive components of Curcuma species, including turmeric (Curcuma longa L.), are curcuminoids, including curcumin, demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) (Meng et al., 2018). Turmeric, an Indian spice, contains curcumin, a class of polyphenols. Turmeric contains a significant amount of a naturally occurring yellow pigment that is frequently referred to as "Indian solid gold" (Aggarwal et al., 2007).

For centuries, curcumin has primarily been used in the food industry because of its vibrant color. However, the pharmaceutical industry has recently shown great interest in this compound due to its various pharmacological effects, such as its ability to fight cancer (Tamaddoni et al., 2020), reduce cellular aging (Aggarwal et al., 2007), antioxidant (Abrahams et al., 2019), anti-inflammatory (Zhou et al., 2020), anti-diabetic (Pivari et al., 2019), antibacterial, antiviral, and antifungal benefits (Zorofchian et al., 2014).

The World Health Organization (WHO) has recommended quality control of herbal medicines to ensure the identification of plant materials, purity, and content (Baghel et al., 2017). The identification and characterization of secondary metabolites in plants pose a challenge in the fields of chemistry and pharmacology, as these compounds are typically present in very small quantities and possess complex molecular structures (Hadacek, 2022).
Therefore, the development of advanced and sensitive analytical techniques is necessary for identifying and characterizing secondary metabolites in plants (Simmler et al., 2014).

There is several instrumental technique to determine of curcumin compound such as ultraviolet-visible spectroscopy (UV/Vis) (Emre Unsal et al., 2019), isocratic high-performance liquid chromatography (HPLC), high-performance liquid chromatography with ultraviolet detection (HPLC-UV) (Khoshidi et al., 2020) and fluorescence (HPLC-FLD) (Schiborr et al., 2010), ultra-high-performance liquid chromatography-mass spectrometry (UPLC-MS) (Sandhiutami et al., 2021), reversed-phase liquid chromatography (RPLC) (Rodriguez et al., 2021), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Song et al., 2019) ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) (Yu et al., 2019), capillary electrophoresis with amperometric detection (CE-AD) (Sun et al., 2002), electrochemical sensors (Raril et al., 2020) and supramolecular solvent-based liquid-liquid microextraction (SMS-LLME) (Caleb & Alshana, 2021).

Analytical methods have been developed to detect curcumin, and each method has its own advantages and limitations (Dey et al., 2018). For instance, spectroscopic methods can only provide the total curcuminoid content, whereas other advanced methods can simultaneously quantify individual curcuminoids (Kadam et al., 2013). Therefore, selection of the analysis method is crucial for obtaining better results. Many critical reviews are available on the chemical, biological, and pharmacological properties of curcuminoids. However, reviews specifically focusing on various curcuminoid analysis methods are limited. Quantitative analysis plays a crucial role in providing precise information regarding the amount of curcuminoids in a sample. This is essential for measuring the levels and distribution of curcuminoids in various matrices such as food ingredients, supplements, or pharmaceutical products containing curcumin. Appropriate analysis methods can yield accurate data to assess product effectiveness and consistency, support scientific research, and ensure compliance with the established standards. This review article aims to provide an overview of several analytical methods for the determination of curcumin. It is hoped that this will encourage readers to choose an appropriate analytical method for their specific needs.

RESEARCH METHOD
The articles used in this review were sourced from international English-language articles obtained from ScienceDirect, PubMed, and Google Scholar. The search was conducted using keyword “Identification curcumin compound or Determination curcumin compound”, “Analytical techniques for curcumin analysis”

DISCUSSION
Curcuminoids are phenolic compounds consisting of three derivatives of curcumin: curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The physicochemical properties of curcuminoids can influence the analytical methods used to measure their concentration and identify these compounds in the samples (Meng et al., 2018).

Curcumin was first discovered by Vogel and Pelletier in 1815 by isolating this substance from the rhizomes of C. longa. In 1842, Vogel Jr. performed the first purification of curcumin. After several decades, in 1910, Melabedzka et al. reported the structure of curcumin as diferuloylmethane, specifically 1, 6-heptadiene-3, 5-dione–1, 7-bis (4-hydroxy–3-methoxyphenyl) (1E,6E) Figure 1 (Prasad et al., 2014). In 1913, Lampe and Melobedzka reported a method for synthesizing curcumin. In 1953, Srinivasan reported the separation and quantification of curcumin components using chromatographic techniques. (Gupta et al. 2012; Prasad et al. 2014; Priyadarsini 2014).
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One of the physicochemical properties of curcumin is its solubility in organic solvents, such as ethanol, methanol, and acetone. Therefore, extraction methods using organic solvents can be employed to separate and measure the concentration of curcumin in samples. Additionally, this property allows high-performance liquid chromatography (HPLC) with a mobile phase consisting of a mixture of organic solvents and water to separate and measure the concentration of curcumin in samples (Lee & Choung, 2011).

Another physicochemical property of curcumin is its ability to absorb light at specific wavelengths. Therefore, UV-Vis spectroscopy can be used to measure the total concentration of curcumin in a sample. This property also enables the use of infrared spectroscopy (IR) to identify curcumin in samples. The physicochemical properties of curcumin influence its stability and bioavailability. Therefore, curcumin analysis methods should consider these factors to ensure the accuracy and reliability of analytical results (Kadam et al., 2013).

In addition, chromatographic methods such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) can be used for curcuminoid analysis. These methods enable the separation of closely related curcuminoid components and can be used to identify and measure the concentration of curcuminoids in the samples. Overall, spectroscopy and chromatography are useful methods for analyzing curcuminoids in food and pharmaceutical samples. However, the choice of method depends on the analytical objectives and properties of the sample to be analyzed.

In a study conducted by Taylor and McDowell in 1992, a comparison was made between spectrophotometric and HPLC methods for the analysis of turmeric rhizomes. The results of this study showed that the spectrophotometric method yielded a higher curcumin content than the HPLC method. This is due to the fact that non-curcumin components can also be absorbed at the same wavelength as curcumin (Chromatography and Words 1992).

Sensitive, accurate, and robust analytical methods are required to quantify curcumin because of: a) its low concentration in plasma/serum/tissues resulting from low bioavailability, rapid metabolism, and degradation; b) matrix interference; and c) appropriate evaluation of bioefficacy and understanding of its mechanisms/mode of action (Alves et al., 2019). The instruments used for curcumin analysis had different
detection limits. A lower detection limit indicated that the analytical instrument was more sensitive for the detection of curcumin in samples. Therefore, an analytical instrument with a lower detection limit is more suitable for analyzing curcumin in samples with low concentrations.

Table I. Lowest Detection Limits of Instruments in Curcumin Analysis

<table>
<thead>
<tr>
<th>Method</th>
<th>Lowest Detection Limits of Instruments</th>
<th>Description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Near-infrared spectroscopy</td>
<td>10 ng/mL</td>
<td>In this study, researchers developed a rapid and non-destructive quantification method for curcuminoids in turmeric using near-infrared spectroscopy (NIR) and multivariate statistics. Turmeric samples were measured with NIR spectroscopy in the range of 1500-2500 nm, excluding the water absorption range (1850-2040 nm). The acquired spectrum data was then processed using pre-processing techniques such as SNV and second derivative. Subsequently, partial least squares (PLS) regression analysis was employed to build a quantification model for curcuminoids in turmeric.</td>
<td>(Tanaka et al., 2008)</td>
</tr>
<tr>
<td>HPLC-fluorescence method</td>
<td>15 ng/ml</td>
<td>This method utilizes a simple ultrasonic extraction technique with methanol as a pretreatment for turmeric products. The curcuminoids and 2,5-xylenol (internal standard) were effectively separated within a 30-minute timeframe using a Cadenza CD-C18 column (250 x 4.6 mm; i.d., 3 mm) and a mixture of acetate buffer and CH3CN. The calibration curve, based on standard curcuminoids, exhibited excellent linearity with a correlation coefficient exceeding 0.993. The instrumental detection limits for curcumin, demetoksi kurkumin, bisdemetoksi kurkumin (at a signal-to-noise ratio of 3) were 1.5, 0.9, and 0.09 ng mL⁻¹, respectively. The relative standard deviations obtained from intra-day and inter-day assays, which involved the addition of curcuminoids to turmeric powder, were below 6.1%.</td>
<td>(Zhang et al., 2009)</td>
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<tr>
<td>Method</td>
<td>LOD (μg/mL)</td>
<td>Details</td>
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<tr>
<td>UPLC</td>
<td>40.66</td>
<td>The method underwent validation in accordance with the ICH guidelines for analytical procedure validation, encompassing precision, accuracy, and linearity. The respective limits of detection for C, DMC, and BDMC were 40.66 pg, 49.38 pg, and 29.28 pg. For quantitation, the limits were determined as 134.18 pg, 164.44 pg, and 97.50 pg for curcumin, demetoksi kurkumin, and bisdemetoksi kurkumin, respectively. The linear range spanned from 3.28 to 46.08 g/ml. The average percent recoveries ± SD of curcuminoids were calculated as 99.47 ± 1.66, 99.50 ± 1.99, and 97.77 ± 2.37 for C, DMC, and BDMC, respectively. To assess system performance, a comparison was made with conventional HPLC in terms of analysis time, efficiency, and sensitivity. (Cheng et al., 2010)</td>
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<tr>
<td>UV–VIS spectrophotometric method</td>
<td>39</td>
<td>The developed method demonstrated the best results in terms of linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) for the standard laboratory mixtures of pure compounds and methanolic extracts of the drugs. The linear range for curcumin (CUR) and berberine (BER) was found to be between 2 and 8 μg mL⁻¹ and 6 and 12 μg mL⁻¹, respectively. The LOD values for curcumin (CUR) and berberine (BER) were 0.039 μg mL⁻¹ and 0.025 μg mL⁻¹, respectively, while the LOQ values were determined to be 0.13 μg mL⁻¹ for curcumin (CUR) and 0.08345 μg mL⁻¹ for berberine (BER). (Pundarikakshudu &amp; Dave, 2010)</td>
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<td>LC–MS/MS</td>
<td>1</td>
<td>This method demonstrated linearity from 1 to 1000 ng/mL with a lower limit of quantification of 1 ng/mL in cell medium, and 5 ng/mL in mouse plasma for all tested curcuminoids. The within-day coefficients of variation were found to be below 15%, and the accuracy ranged from 85% to 115%. (Vijaya Saradhi et al., 2010)</td>
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<tr>
<td>HPTLC</td>
<td>49</td>
<td>The HPTLC separation was performed on an aluminum-backed HPTLC plate with a layer thickness of 0.2 mm using silica gel 60 F254 and a mobile phase consisting of a combination of dichloromethane and methanol (99:1). (Gantait et al., 2011)</td>
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The plate was developed up to 80 mm at a temperature of 20 ± 4°C with a chamber saturation time of 10 minutes. Under these conditions, the retardation factor (Rf) of curcumin was found to be 0.43, and the compound was quantified at its maximum absorption wavelength (λ max) at 427 nm. The limits of detection and quantification were determined to be 49 ng and 148 ng per spot, respectively. The response of curcumin was linear within the range of 0.8 µg to 1.3 µg per spot, with a correlation coefficient of 0.99395 indicating a good relationship between peak area and concentration.

The three curcuminoids were effectively separated on a C18 column and detected with high sensitivity. The mobile phase used consisted of acetonitrile and 10 mm Na2HPO4–H3PO4 (pH 5.0) (50:50, v/v). Good linearity was achieved in the ranges of 0.208–41.6, 0.197–39.4, and 0.227–114 µM for curcumin, demethoxycurcumin, and bisdemethoxycurcumin, respectively. The detection limit reached up to 10⁻⁸ M, which was lower than that of UV detection. The relative standard deviations (RSDs) ranged from 1.06% to 1.88% for intra-day precision and from 4.30% to 5.79% for inter-day precision. The proposed method has been successfully applied to real plant samples, and the recoveries ranged from 86.3% to 111%.

In this study, a rapid, selective, efficient, and reproducible HPLC method for the separation and identification of curcuminoids using a Sunniest PhE (phenyl) column with dimensions of 250 x 4.6 mm and a particle size of 5.0 mm was described. The components were successfully separated within 10.5 minutes using a mobile phase consisting of a mixture of acetonitrile-methanol-water (40:20:40, v/v) at a flow rate of 1.0 mL/min and detected at a wavelength of 360 nm. The capacity factors (k, 4.2 to 4.9), separation factors (a, 1.07 to 1.10), and resolution factors (Rs, 1.07 to 2.05) indicated a good separation of the
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compounds. The reported method is considered as a novel approach as it achieved baseline separation of curcuminoids with clear peaks and low detection limits compared to previously reported methods in the literature.

In this study, a micellar electrokinetic chromatography (MEKC) method using a mixture of micelles composed of Triton X-100 and SDS was employed to enhance the natural fluorescence of curcuminoids and improve their separation efficiency. The fluorescence spectrum analysis results revealed that the synergistic fluorescence induced by the mixed micelles could increase the signal of the three curcuminoids by 77-fold for curcumin, 57-fold for demetoksi kurkumin, and 47-fold for bisdemetoksi kurkumin. Through systematic investigation, it was found that the optimal separation buffer for curcuminoids consisted of 20 mM Triton X-100, 20 mM SDS, 30% (v/v) methanol in a 10 mM borax solution at pH 10.0. Under these conditions, a baseline separation of the three curcuminoids was achieved within 10 minutes, and the detection limits were determined to be 4.1 ng/mL for curcumin, 2.6 ng/mL for demetoksi kurkumin, and 0.4 ng/mL for bisdemetoksi kurkumin. (Wu et al., 2018)

Spectrophotometric methods

The basic principle of spectroscopy is that chemicals absorb light at specific wavelengths. When light passes through a sample, some wavelengths of light are absorbed by molecules in the sample, whereas others are transmitted. This absorption spectrum can be used to identify and measure the concentrations of chemicals in a sample.

Spectroscopy can be performed at various wavelengths of light such as ultraviolet (UV), visible (Vis), and infrared (IR). In UV-Vis spectroscopy, light of the desired wavelength is passed through the sample, and the intensity of the transmitted light is measured. In IR spectroscopy, infrared light passes through the sample, and the resulting absorption spectrum is used to identify and measure the concentration of chemicals in the sample.

Several spectroscopic techniques, such as Fourier Transform Infrared (FT-IR) spectroscopy, near-infrared (NIR) spectroscopy, Raman spectroscopy, and ultraviolet/visible (UV-Vis) spectroscopy, have been used for the qualitative and quantitative analysis of curcuminoids and commonly found additives. In a study
conducted by Dhakal et al. (2016), FT-IR and Raman spectroscopy were used to identify additives such as metanil yellow and Sudan-I in the turmeric powder. The results of this study showed that FT-Raman exhibited higher sensitivity than FT-IR in detecting metanil yellow in turmeric.

Tanaka et al., (2008), utilized near-infrared (NIR) combined with multivariate analysis to quantify curcuminoids in Curcuma rhizomes and compared the results with HPLC quantification. The authors observed that prediction using partial least squares regression showed a high correlation with the HPLC quantification results. Curcuminoids exhibit strong UV-Vis absorption at λmax 425 nm (Priyadarsini, 2014) and UV-Vis spectroscopy can easily be employed for curcuminoid quantification if the sample matrix or other coexisting components do not absorb within this range (Tanaka et al., 2008).

The main advantages of spectroscopy-based analysis are its speed, ease, and cost efficiency. However, these techniques are not as sensitive as mass spectrometry and are often susceptible to matrix interferences with components showing absorption at the same wavelength as the analyte. Spectroscopic methods (UV, IR, and NIR) can be used as high-throughput screening tools. All spectroscopic techniques are influenced by the matrix and often require confirmation using specific detection techniques (HPLC or HPLC combined with mass spectrometry).

Chromatographic methods

HPLC has been widely used to quantify analytes in various fields. HPLC can be coupled with different detectors such as UV, fluorescence, and mass spectrometry (MS). Although each detector has its own advantages and limitations, MS has proven to be a highly useful, sensitive, accurate, and robust detector. For the separation of curcumin using HPLC, most studies have employed reversed-phase liquid chromatography, particularly C18 columns with various particle sizes. Although most reports are based on C18 columns, C8 columns and other columns have also been used for curcumin separation.

HPLC (High-Performance Liquid Chromatography) can be used for curcumin testing because HPLC is a highly sensitive and accurate liquid chromatography technique for separating, identifying, and measuring compounds in mixtures. HPLC utilizes a column filled with a stationary phase and mobile phase to separate compounds in the mixture based on their physicochemical properties (Setyaningsih et al., 2021).

HPLC, apart from other liquid chromatography instruments, has a high flow rate of the mobile phase, allowing for the separation of compounds in a relatively short time. Additionally, HPLC provides better resolution and higher sensitivity than other liquid chromatography techniques. Thus, HPLC is the preferred choice for analyzing complex compounds such as curcumin in microparticle formulations (Setyaningsih et al., 2021).

The method for estimating curcumin and its related components was first used by Tønnesen and Karlsen in 1983. Jayaprakasha et al., (2002), made modifications to the existing HPLC protocol by introducing a gradient elution technique using a solvent mixture consisting of acetonitrile, methanol, and acetic acid. This modified method enabled the determination of curcuminoids within a 20-minute run time.

In recent years, there have been numerous reports of improved and validated HPLC methods following the guidelines of the International Conference on Harmonization (ICH) (Ali et al., 2014; Wichitnithad et al., 2009). However, most HPLC methods described in the literature for quantifying curcuminoids have encountered various limitations. These limitations include high flow (Osorio-Tobón et al., 2016), lengthy analysis times (R. Li et al., 2011) complex gradient elution (F. Li t 2014), the use of buffer solutions in the mobile phase, and high detection limits (Jangle & Thorat, 2013). Furthermore, these methods are often restricted to the measurement of curcuminoids in specific turmeric products (Peram et al., 2017). Consequently, researchers are currently focusing on improving HPLC methods to enable rapid identification, separation, and measurement of curcuminoids (Lechtenberg et al., 2004). Nevertheless, efforts to address these limitations are still ongoing.
In their study Jia et al., (2017) employed the UHPLC-QTOF-MS/MS (Ultra-High Performance Liquid chromatography quadrupole time-of-flight tandem mass spectrometry) technique to analyze curcuminoids in turmeric rhizomes. The research findings indicate that this method allows for the separation of isomers within the same category of curcuminoids, a capability not achievable with other chromatographic techniques. Despite the simplicity, cost-effectiveness, and rapidity of the UHPLC method and its recognition as the most dependable approach for curcuminoid analysis in quality control, there is a scarcity of literature reporting on the application of UHPLC.

**Liquid chromatography-mass spectrometry (LC–MS)**

LC-MS/MS serves as a robust analytical instrument for sample analysis and offers both qualitative and quantitative insights. The tandem mass spectrometry technique delivers precise structural details of the analyte, allowing for accurate measurements even at extremely low concentrations (ranging from nano-to picogram/mL) (Huang et al., 2019). However, LC-MS/MS instruments come at a high cost and demand a substantial level of technical proficiency and diligent upkeep. The selection of a sample analysis technique depends on instrument availability and the research objectives.

He et al. (1998) reported the first LC-MS method for curcuminoid analysis in turmeric rhizomes. In this study, they utilized electrospray ionization mass spectrometry and UV diode-array detection to analyze curcuminoids; however, complete structural details were not reported. This was because they used a one-dimensional LC-MS method with only one ionization mode. Therefore, the use of tandem mass spectrometry can overcome this limitation. The fragmentation behavior of the three curcuminoids in an LC-MS/MS ion trap was investigated by Jiang, Somogyi, et al., (2006), in the positive and negative electrospray ionization modes. They also employed off-resonance irradiation (SORI) in a Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometer. In this study, the three curcuminoids in turmeric rhizomes were identified together with other minor curcuminoids, and their origins were determined. Jiang, Timmermann, et al., (2006) used LC-ESI-MS/MS combined with Diode Array Detection (DAD) to identify known and unknown diarylheptanoids in fresh turmeric rhizome extracts and identified 12 new diarylheptanoids.

In recent years, there has been an increasing demand for rapid and sensitive analytical methods to quantify curcumin at trace levels, driven by a growing number of animal studies and clinical trials investigating its effectiveness as a therapeutic agent. Consequently, several LC-MS/MS-based methods have been developed and validated, taking advantage of the superior sensitivity and accuracy of the MS detectors. For example, Huang et al. (2019) recently published a validated LC-MS/MS method capable of quantifying curcumin and other compounds, exhibiting an outstanding lower limit of quantification (LLOQ) of 0.01 ng/mL (10 pg/mL) and low limit of detection (LOD) of 0.004 ng/mL (4 pg/mL). Similarly, Ahmad et al. (2016) reported a validated LC-MS/MS method with an LLOQ of 0.05 ng/mL and an LOD of 0.02 ng/mL for measuring curcumin in rat brain homogenates and plasma.

**CONCLUSION**

The spectrophotometric method is a simple technique used to estimate curcumin content, but its usefulness is limited to cases where the concentration of individual curcuminoids is not a critical quality parameter. Infrared spectrometry, on the other hand, is a fast and nondestructive method suitable for quality control in industrial settings. Prior separation of curcuminoids using TLC allows the identification of low levels of curcuminoids without interference from other compounds. However, optimizing the mobile phase, spot width, and plate-to-plate variation presents significant challenges in the TLC and HPTLC methods. There are numerous research reports available for estimating curcuminoid content using HPLC, although these methods strongly depend on the type of the sample matrix. LC-MS/MS is the preferred choice to accurately determine very low levels of curcuminoids in various sample matrices. The precise quantification and detection of trace...
amounts of curcuminoids and metabolites require the use of chromatographic separation methods combined with mass spectrometry detection (LC-MS/MS), as they offer high accuracy, reproducibility, and sensitivity with low limits of detection (LOD) and lower limits of quantification (LLOQ).

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