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Note

High-performance liquid chromatography of curcumin and related compounds

HANNE HJORTH TØNNESEN* and JAN KARLSEN
Department of Galenical Pharmacy, Institute of Pharmacy, University of Oslo, Oslo (Norway)
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The rhizomes of the plant *Curcuma longa* L. (*Zingiberaceae*) (*Turmeric*) have a traditionally important rôle as a colouring agent in food, cosmetics and textiles. Today turmeric, turmeric extracts and turmeric oleoresins are commercial products produced in large quantities (160,000 tons per year)[1–3]. With the demands for natural colours, the use of turmeric is likely to increase. It is therefore of interest to develop an high-performance liquid chromatographic (HPLC) method by which the coloured compounds of *Curcuma longa* L. can be quantitatively analysed in small amounts as pure compounds or in commercial products.

The main coloured substances in the rhizomes of *Curcuma longa* L. are curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], and two related demethoxy compounds, demethoxycurcumin and bisdemethoxycurcumin (Fig. 1)[4]. These compounds belong to the group of diarylheptanoids[5,6]. Not too much is known about the stability and analysis of the curcuminoids. A variety of methods for their determination have been published. Usually, separation of the curcuminoids is achieved by thin-layer or paper chromatography[1,2,7–10], often in combination with spectroscopy for quantitative examination. If necessary, a more intensely coloured complex can be developed by reaction with boric acid to lower the detection limit[11–15]. The boric acid test is one of the official identification tests for curcumin and turmeric requested by WHO/FAO[16,17]. An alternative detection method for the coloured compounds in *Curcuma longa* L. is based on the fluorescent property of tumeric. The curcumin content in a sample can be determined with a spectrofluorimeter after an extraction process[18–20].

For an accurate determination of curcumin the described methods are often

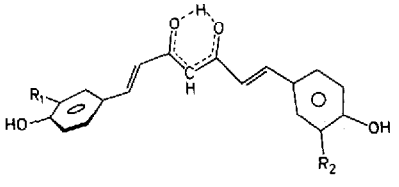

Fig. 1. The main coloured substances of *Curcuma longa* L.

$R_1 = R_2 = OCH_3$	Curcumin
$R_1 = OCH_3$, $R_2 = H$	Demethoxycurcumin
$R_1 = R_2 = H$	Bisdemethoxycurcumin

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unsatisfactory. The boric acid test is reported to give inconclusive results because of interference from co-extractives[20]. We have investigated[21] the coloured compounds in turmeric by thin-layer chromatography (TLC) and always observed a yellow compound with $R_F = 0$ on silica gel in addition to the three compounds separated. This may give rise to incorrect results when TLC is used for quantitative analyses. The fluorimetric test is reported to be unsuitable for quantitation of an unknown product containing curcumin (turmeric)[20]. An HPLC method for the determination of curcumin has been reported[22]. This chromatographic system, however, does not separate the three curcuminoids.

We have developed a simple HPLC system which separates curcumin and its structural isomers, and makes it possible to determine with high accuracy the absolute curcumin content of a sample.

EXPERIMENTAL

Curcumin, demethoxycurcumin and bisdemethoxycurcumin were analysed by HPLC. Standard curves for the three isomers were constructed.

Reagents

The curcuminoid standards were synthesized and tested by mass spectrometry (MS) and TLC. Pure, natural curcumin was donated by Chr. Hansen's Laboratory, Denmark. Natural, crystalline curcumin was supplied by Koch-Light (Colnbrook, Great Britain). Plant material from *Curcuma longa* L. and *Curcuma zedoaria*, Roscoe were obtained from Norsk Medisinaldepot, Norway. The chemicals used were of p.a. grade.

Preparation of standard solutions

1-mg amounts of each of the synthetic curcuminoids were dissolved in methanol to give 50-ml stock solutions. From this, dilutions in methanol were made. Standard curves from 1 to 20 ng/μl were prepared for UV–visible detection. For fluorescence detection, standard curves in the ranges 1–20 ng/μl, 0.5–5 ng/μl and 0.05–1 ng/μl were constructed for curcumin, demethoxy- and bisdemethoxycurcumin respectively.

Chromatographic conditions

A Spectra-Physics Model 3500 high-performance liquid chromatograph was used. The UV–visible detector was a LDC Spectro Monitor III, and the fluorescence detector was a Schoeffel L.C. Fluorometer FS 970. The stationary phase was Nucleosil NH_2 (Chrompack), particle size 5 μm, pre-packed in a 250 × 4.6 mm I.D. column. The mobile phase was ethanol, flow-rate 1.2 ml/min. A Rheodyne injector with a 20-μl loop was used. The analyses were carried out at ambient temperature. Detection conditions: a, UV, 254 nm; b, visible, 420 nm; c, fluorimetric, excitation wavelength 420 nm, emission wavelength 470 nm.

RESULTS

The linearity of the method was tested for each of the curcuminoids when

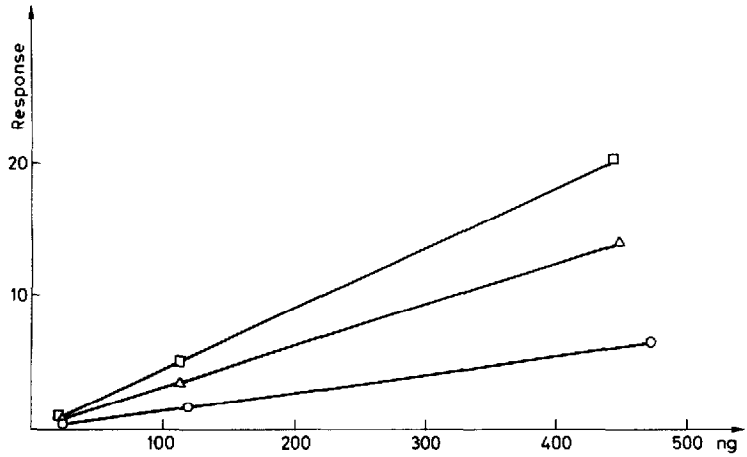


Fig. 2. Standard curves of the three curcuminoids. O, Curcumin, $y = 0.013\ x + 0.086$ ($r = 0.99$); △, demethoxycurcumin, $y = 0.031\ x - 0.035$ ($r = 0.99$); □, bisdemethoxycurcumin, $y = 0.045\ x - 0.189$ ($r = 0.99$). Detection at 420 nm.

UV (254 nm), visible (420 nm) and fluorescence detection were used. A linear relationship ($r = 0.99$) was observed for each of the curcuminoids and with each detection method over the ranges studied. Standard curves are shown in Figs. 2 and 3.

A typical chromatogram obtained from a sample of natural curcumin (fluorescence detection) is shown in Fig. 4. This indicates that all the three curcuminoids are present in commercially available curcumin. The detection limits for the isomers are below 20 ng using a UV-detector. A ten-fold increase is obtained by using fluorescence detection. This method is suitable for the determination of curcumin and its related compounds in a variety of products (plant material, food products). *Curcuma zedoaria*, Roscoe is reported not to contain bismethoxycurcumin[1,2,7,8,18]. An

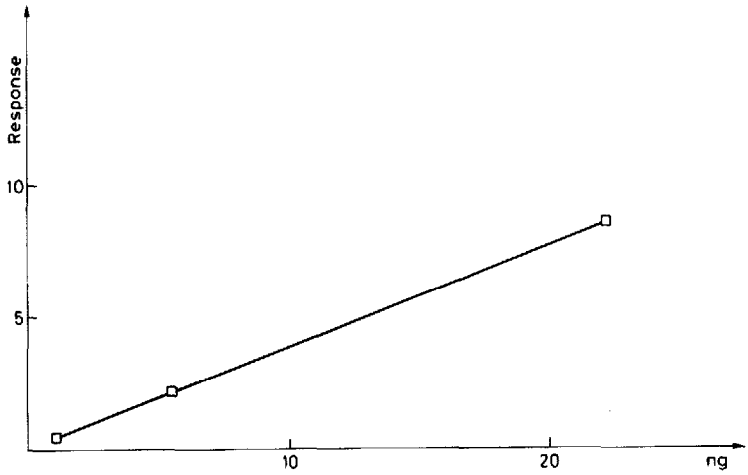


Fig. 3. Standard curve of bisdemethoxycurcumin. $y = 0.383\ x + 0.045$ ($r = 0.99$). Fluorescence detection; exitation wavelength 420 nm, emission wavelength 470 nm.

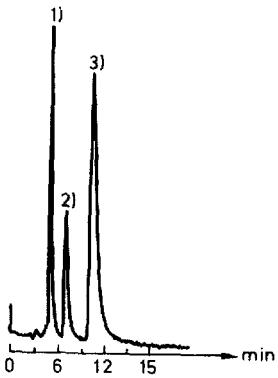


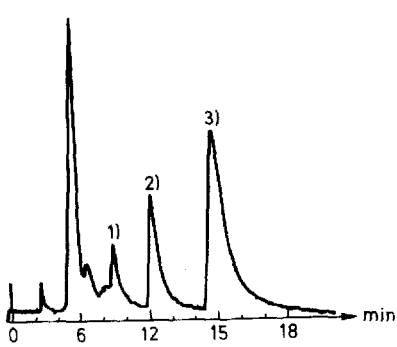
Fig. 4. The three curcuminoids separated on a Nucleosil NH_2 column, using fluorescence detection. Peaks: 1 bisdemethoxycurcumin; 2 demethoxycurcumin; 3 curcumin.

HPLC analysis using fluorescence detection, however, indicated that the bismethoxy isomer is present in this plant material. (Fig. 5).

DISCUSSION

The HPLC method described gives a baseline separation of the three curcuminoids. The analytical procedure has a high sensitivity, and the use of fluorescence detection gives a very low detection limit. An example is shown in Fig. 6. Amounts down to $2 \cdot 10^{-10}$ g ($5.57 \cdot 10^{-13}$ moles) of curcumin, $1.67 \cdot 10^{-10}$ g ($3.45 \cdot 10^{-13}$ moles) of demethoxycurcumin and $1 \cdot 10^{-12}$ g ($3.25 \cdot 10^{-15}$ moles) of bisdemethoxycurcumin can be measured.

By comparing the slopes of the standard curves for the curcuminoids obtained with the same detection method and for each curcumin isomer using different detection methods we found that: (1) at 254 nm the demethoxy isomers have twice the absorption of curcumin; (2) at 420 nm the proportions are 1:2.4:3.5 (curcumin:demethoxycurcumin:bisdemethoxycurcumin) and (3) with fluorescence detection the proportions are 1:2.2:10.4. If we look at curcumin, the response increases from 254



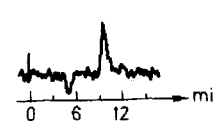


Fig. 5. Typical chromatogram of extract from *Curcuma zedoaria*, Roscoe, using fluorescence detection Peaks as in Fig. 4.

Fig. 6. The fluorescence response obtained by injecting 20 pg bismethoxycurcumin.

nm to 420 nm and fluorescence detection in the ratio 1:4.3:61.6. The corresponding values for demethoxy- and bisdemethoxycurcumin are 1:5.2:66.7 and 1:7.1:294.6 respectively. This shows that fluorescence detection is by far the most sensitive detection method for the curcuminoids in this HPLC system. Further, it underlines the importance of separation of the isomers before quantitation.

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