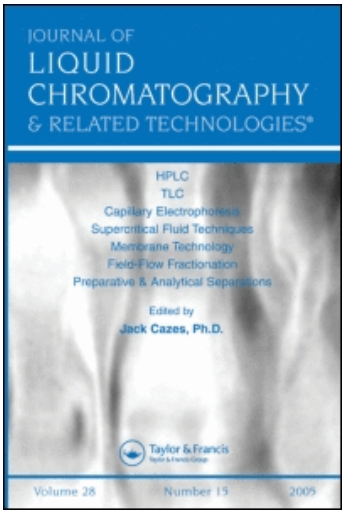

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ISOLATION OF GINGEROLS FROM POWDERED ROOT GINGER BY COUNTERCURRENT CHROMATOGRAPHY

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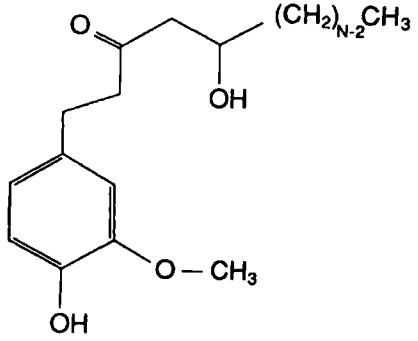
ABSTRACT

A rapid process has been developed for the isolation of [6]-, [8]- and [10]-gingerols, in quantities between 40mg to 500mg, from powdered root ginger using countercurrent chromatography. Minor modifications to the procedure allowed the separation of [4]- gingerol. Optimisation of the CCC technique also led to the development of a normal phase HPLC system using a diol-bonded column eluted with the less polar phase of a typical countercurrent system. This system resolved the gingerols present in a crude methanol extract and gave good separation from potentially interfering constituents.

INTRODUCTION

Gingerols are an homologous series of phenolic ketones present in the rhizomes of ginger (*Zingiber officinale*), the major components of which are responsible for the pungency of ginger [1]. [6]-, [8]- and [10]- Gingerols form the major constituents and a further five homologues, [3]-, [4]-, [5]-, [12]- and [14]-gingerols [2,3] have been reported to occur naturally. Ginger itself has long been

used as a flavouring agent, carminative and stimulant and more recently, the gingerols have been shown to exhibit a number of pharmacological effects including inhibition of prostaglandin biosynthesis [4-6], anti-hepatotoxic [7] and cardiotonic [8]. This latter effect is, more specifically, attributable to activation of the Ca^{2+} -pumping ATPase in cardiac sarcoplasmic reticulum [8-10].



[N]- Gingerol

Gingerols have been analysed by thin layer chromatography on silica [3] and by HPLC on reversed phase columns [3,11-14] Preparative separation techniques have included multiple step open-column chromatography over silica gel [8] or HPLC using reverse phase columns [3]. Both of these procedures have proved to be time consuming and the latter has been reported to give products, especially [6]- gingerol, of limited purity [14]. Since we required to isolate gram quantities of individual gingerols for pharmacological evaluation, we decided to investigate an alternative separation method, based upon countercurrent chromatography.

High speed countercurrent chromatography (CCC) is a technique, where a liquid stationary phase is retained within a multilayer coil that is subjected to a fluctuating field of centrifugal forces, while an immiscible mobile phase in equilibrium with the stationary phase is pumped through [15]. A sample injected into the mobile phase is thus subjected to a large number of partition steps before it is eluted from the coil. One of the major advantages of CCC is that the solvent system used can be selected on the basis of simple partition experiments. The technique has proved very effective in the isolation of compounds of pharmaceutical interest from natural sources [15-17].

MATERIALS AND METHODS

Reagents

Reagents were of analytical grade except for hexane which was of HPLC grade (HiperSolv, BDH). Powdered Jamaican root ginger was purchased from Potter's Herbal Supplies (Wigan, England).

Countercurrent Chromatography.

CCC was performed using an horizontal flow-through planet centrifuge fitted with an Ito Multilayer Coil comprised of 70m of 2.6mm i.d. PTFE tubing (PC Inc.). Phases were pumped using a Gilson Model 303 pump fitted with a 50.S pumping head and a Model 804C manometric module. Samples were injected via a simple T-piece fitted with a 3-way tap at the "tail" end of the column. The eluant was monitored continuously at 282nm using a Waters 490 UV detector fitted with a semi-prep. cell and fractions were collected using a Pharmacia Frac 100 fraction collector. The absorbance of individual fractions was measured at 282nm using a Perkin-Elmer Lambda 7 UV/VIS spectrophotometer after dilution with methanol .

Appropriate volumes of solvents were mixed thoroughly in a separating funnel and the 2 phases allowed to separate. The column was filled with lower phase by pumping at a flow rate of 20mL.min^{-1}. The sample in 8mL of a mixture of the 2 phases was injected via the T-piece, the centrifuge operated at 800 rev.min^{-1} and the upper phase pumped at 3 mL.min^{-1} from the "tail" to the "head" end of the column. Retention of the stationary was greater than 90%. At the end of the run the centrifuged was turned off and the stationary phase displaced from the column with methanol at a flow rate of 20mL.min^{-1}.

HPLC

HPLC was carried out using a diol column (5μ spherical packing, 4.6 x 250 mm, J.T.Baker). Solvent was pumped using a Waters 600E system controller fitted with a U6K injection system. A Waters 990 diode array detector coupled to a Waters 820 data system was used on line.

TLC

Merck Kieselgel 60 F_{254} plastic backed TLC sheets were used with a solvent system comprised of toluene:ethyl acetate (7:3). Phenolic compounds were visualised by spraying with 5% ferric chloride in ethanol.

Concentration Steps during Extraction.

Concentration of solutions during extraction was carried out by rotary evaporation (Buchi, Model EL131). Final removal of solvents was achieved by evaporation in a stream of dry nitrogen.

Open Column Chromatography on Silica Gel and Diol-bonded Silica.

Chromatography was carried out using Kieselgel 60 (Merck) with toluene: ethyl acetate (7:3) as mobile phase and with 40μ Bonded Phase-Diol (J.T.Baker) using the upper phase of ethyl acetate: hexane: methanol: water (2:3:3:2).

Mass Spectrometry

Mass spectrometry (MS) was carried out using a Finnigan MAT 8400 mass spectrometer operating in the EI mode at 70eV. Samples of each fraction (2μL) were introduced using a Finnigan MAT moving belt interface.

Nuclear Magnetic Resonance (NMR)

Proton NMR spectra were determined in $CDCl_3$ at 500 MHz using a Bruker AM500 spectrophotometer.

RESULTS AND DISCUSSION

Isolation of [6]- [8]- and [10]- Gingerols.

The extraction scheme for the isolation of [6]-, [8]- and [10]- gingerols is outlined in Table 1. The early stages were based on the methods employed by Shoji et. al.[8]. In the absence of standard markers for gingerols, fractions from the silica column were examined by TLC and by mass spectrometry. The

TABLE 1

Extraction Scheme.

Powdered root ginger (450g)

↓ Extracted with methanol.

Methanol extract

↓ Partitioned between ethyl acetate and water.

Ethyl acetate extract (18g)

↓ Chromatographed on silica gel column (6.5x25cm) using toluene:ethyl acetate (7:3).

9 fractions (A-I)

TLC and MS on fractions E,F,G and H suggested the presence of gingerols.

Fraction F (1.28g)	CCC using ethyl acetate:hexane:methanol: water (2:3:3:2)	Fraction G (1.04g)
↓		↓
[10]- gingerol (225mg)		[10]- gingerol (43mg)
[8]- gingerol (211mg)		[8]- gingerol (57mg)
[6]- gingerol (418mg)		[6]- gingerol (539mg)

presence of peaks at M/Z values of 294, 322 and 350 together with a base peak at 137 in fractions that gave a grey diffuse zone with ferric chloride at Rf 0.31-0.42 suggested that the fractions contained gingerols. The CCC system was developed by determining the partition of material with Rf 0.31-0.42 between the upper and lower phases of a range of solvent mixtures. It was found that ethyl acetate: hexane: methanol: water (1:1:1:1) gave approximately equi-partition. The literature suggested, however, that the major gingerol present would be the [6]-gingerol, the most polar, so the less polar CCC system (2:3:3:2) was used to enable the [10]- and [8]- gingerols to be eluted first. In practice, [10]- gingerol

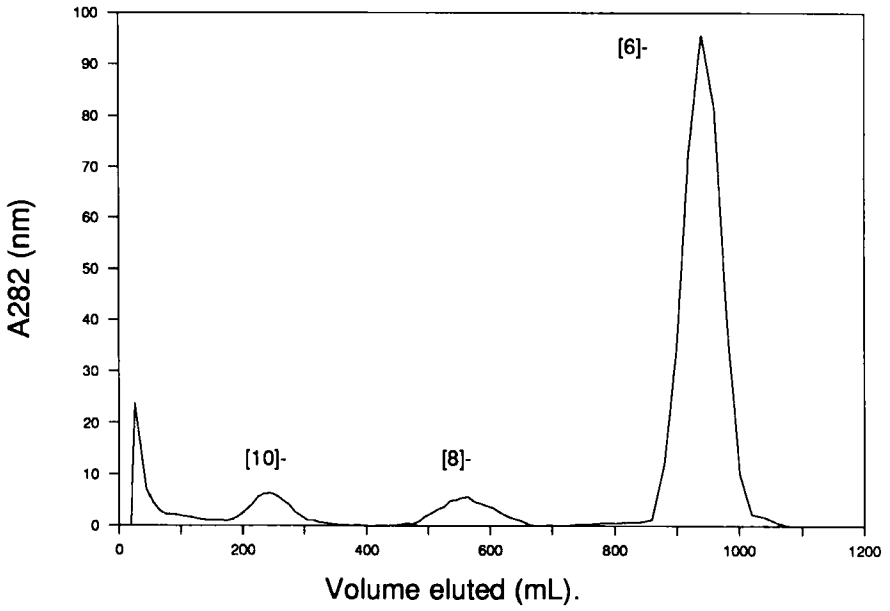


Figure 1. CCC on fraction G (see Table 1). Conditions as in text. After 750 mL had been collected the centrifuge was stopped and the contents of the column displaced with methanol. Gingerol peaks are as indicated.

and [8]- gingerol were eluted during normal elution from the column, whilst [6]-gingerol was retained on the column, being recovered only after unloading the column. An example of a CCC run is shown in Fig. 1.

Isolation of [4]- Gingerol.

A methanol extract from 100g of ginger was processed as in Table 1 except that a more polar CCC system (ethyl acetate: hexane: methanol: water (3:2:2:3)) was used. This system gave incomplete resolution of the major gingerols but gave well-resolved peaks eluting after about 220mL and 300mL. The first of these peaks was dried under nitrogen to give 3mg of a pale yellow oil which was shown to be [4]- gingerol by NMR. The second peak (ca. 1mg) was shown by NMR to be a complex mixture.

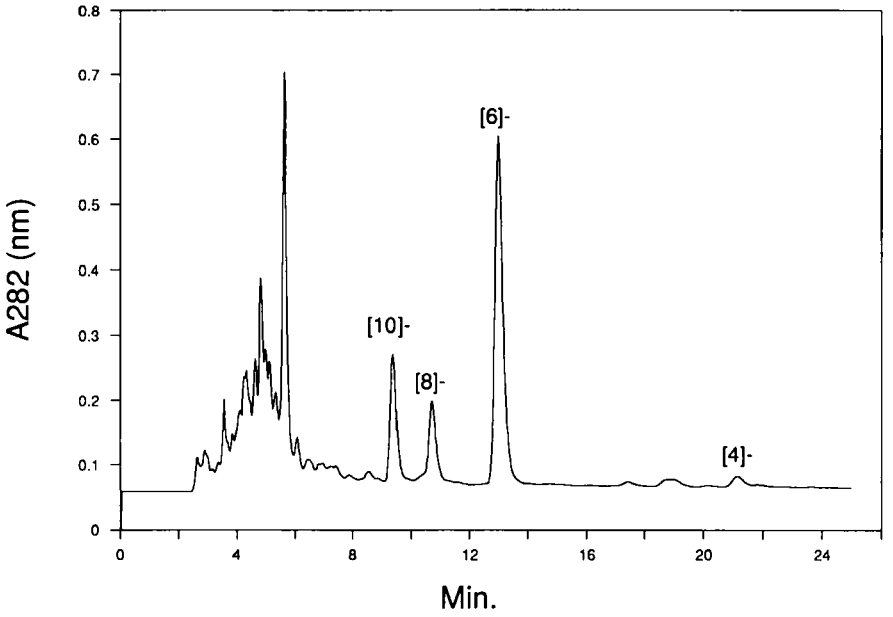


Figure 2. HPLC of a methanol extract of powdered ginger on a 5μ diol column. Conditions as in text. Gingerol peaks are as indicated.

Separations on a Diol-bonded Stationary Phase.

While substantial quantities of gingerols were being prepared as above, some consideration was being given to how the process could be scaled up further in case greater quantities of gingerols were required. Rasmussen and Scherr [18] have demonstrated that separations, broadly similar to those obtained with CCC, can be achieved using open columns of diol-bonded silica and the less polar phase of the CCC system. In principle the scale is only limited by the size of the column. The feasibility of using a diol column for processing ginger samples was examined on an analytical HPLC column using the upper phase of ethyl acetate: hexane: methanol: water (1:2:2:1) at 1mL.min^{-1}. The separation of the gingerols was less than that reported on reverse phase systems, however they were well resolved and there was little interference from other UV absorbing components. This system was used routinely to monitor purity of products. A

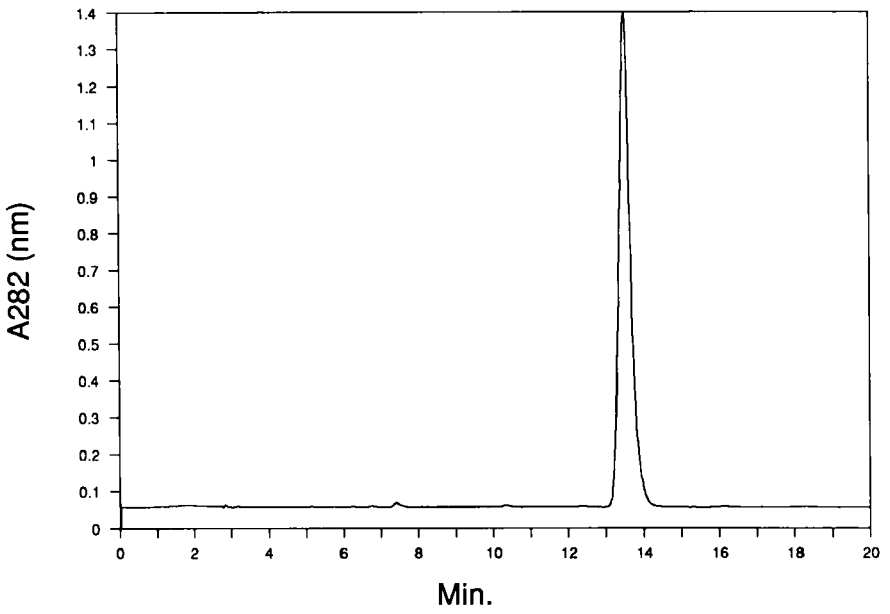


Figure 3. HPLC of [6]- gingerol isolated by CCC. Conditions as in text.

chromatogram of a crude methanol extract of ginger is shown in Fig. 2. When open column chromatography using 40μ diol packing was tried using the CCC mobile phase, the separation was however insufficient to completely resolve the gingerols.

Assessment of Purity of Isolated Gingerols.

The initial assessment of purity of the samples, following CCC, was carried out by proton NMR, which as well as detecting major contaminants confirmed the identity of the individual gingerols. Of the 4 gingerols isolated only [8]-gingerol was shown to have a significant impurity (ca. 10%). HPLC using diol-bonded silica confirmed the presence of this impurity and indicated that the other gingerols were better than 90% pure, the purest being [6]-gingerol at > 97% by UV (see Fig.3).

CONCLUSION

CCC was found to be particularly useful for isolating gingerols from a partially purified extract of ginger on a scale of up to 500mg. The purity of isolated [6]- gingerol at >97% reflects the mild separation conditions of this technique and the potential of CCC in the preparation of samples of high purity suitable for use as analytical standards. Furthermore the high recoveries characteristic of this technique suggest that CCC may well have a more general role in revitalising precious but "tired" standard compounds.

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