

Purification of 4-methyl phenylcoumarins from *Mesua elegans* by Multi Dual Mode Centrifugal Partition Chromatography

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Introduction

Malaysian Clusiaceae includes some well-known important trees and is a family of the main canopy of the forest¹. *Mesua* species have been known to produce various interesting compound structures, which are used for its medicinal values such as treatment of fever, dyspepsia and renal diseases².

Centrifugal Partition Chromatography (CPC) method was developed for the separation of 4-phenylcoumarins from *Mesua elegans* hexanic extract. Comparison study between multiple CPC dual mode and isocratic mode were done for 50, 150, 300 and 500 mg injection.

Methodology

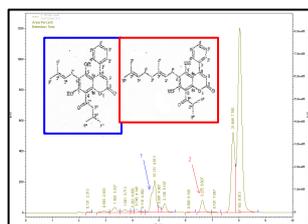


Figure 1: HPLC analysis at 280nm of sample

- Sample (Figure 1) : Fraction F1 from hexane crude of *Mesua elegans* after silica gel column chromatography (230-400 mesh; Hex:EtOAc 9.5:0.5)
- Instrument : Armen SCPC-250 coupled with Armen Spot Prep II
- Solvent : Hept/EtOAc/MeCN/H₂O with 0.1% TFA (9:1:9:1)
- Flow Rate : 10mL/min
- Rotor Speed : 1600 rpm
- Mode of elution : Isocratic or Multiple dual mode



Results and discussion

Two 4-phenylcoumarins compounds were isolated by using CPC (Figures 2 and 3) and the structures of these two compounds were identified by ¹H NMR, ¹³C NMR and LC-MS ; mammea A/BA 1 [(M+H)⁺ = 407.1855] and mesuagenin C 2 [(M+H)⁺ = 461.2777].

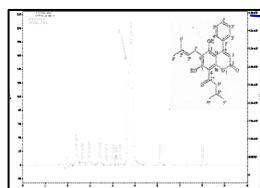


Figure A: HPLC analysis at 280nm of fraction 4

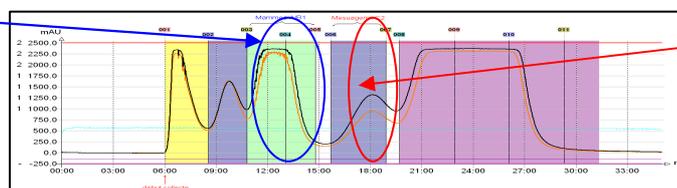


Figure 2: CPC chromatogram of 300mg injection, isocratic mode

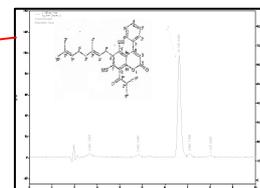


Figure B: HPLC analysis at 280nm of fraction 6

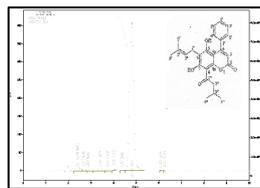


Figure C: HPLC analysis at 280nm of fraction 7

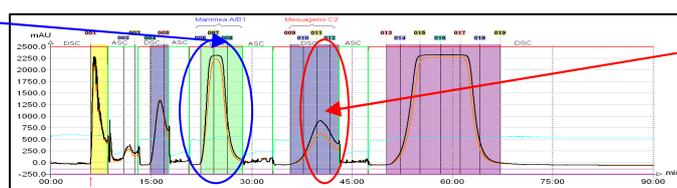


Figure 3: CPC chromatogram of 300mg injection, dual mode

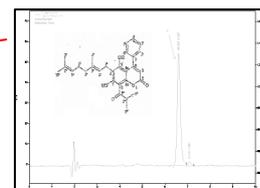


Figure D: HPLC analysis at 280nm of fraction 10

Until 150mg injected, differences of HPLC purity for both compounds between isocratic and multi-dual modes are not significant. But when injected mass are higher, in isocratic mode, the purity decrease below 90% whereas in multi-dual, compounds are up to 93% even when 500mg are injected (see Table 1, Figures A to D).

Mass Injected	Isocratic Mode: DSC		Multi-Dual Mode	
	Mammea A/BA1	Mesuagenin C2	Mammea A/BA1	Mesuagenin C2
50 mg	98.5%	98.1%	99.66%	100%
150 mg	96.2%	94.6%	97.6%	95.5%
300 mg	84.2%	91.1%	93.0%	96.6%
500 mg	82.3%	79.4%	93.2%	94.3%

Table 1: HPLC purity comparison at 293nm of compounds isolated in 300mg injected by isocratic and multi-dual mode

Conclusion

AGP Software allows an automatic variation of ascending to descending mode (and vice versa) for multi-dual CPC separation. By using this elution mode, two compounds had been successfully isolated with high purity.

In this study, optimization of multi-dual mode was done to increase quantity injected for a good ratio recovery/purity.

[1] Whitmore, T.C.(Ed.), (1973), *Tree Flora of Malaya – Volume Two*, p.162-236.

[2] Verotta, L., Lovaglio, E., Vidari, G., (2004), *Phytochemistry*, 65, 2867-2879.

Notes : This application note has been produced and edited using information that was available when the data was acquired for each article. This application note is subject to revision without prior notice