Development of an analytical method for the analysis of monoterpenes in *Alpinia zerumbet* essential oil by gas chromatography using central composite design optimization combined with different column types

Igor C. Cardoso[1,2], Ramon G. Paschoal[1], Henrique Marcelo G. Pereira[2], Maria D. Behrens[1], Marcelo R. R. Tappin[1]

[1] Fundação Oswaldo Cruz - Rua Sizenando Nabuco 100, Manguinhos, Rio de Janeiro, Brazil.
[2] Universidade Federal do Rio de Janeiro - Avenida Athos da Silveira Ramos 149, Rio de Janeiro, Brazil.
igorcunhacardoso@gmail.com

Keywords: *Alpinia zerumbet*, essential oil, central composite design.

Alpinia zerumbet is native of West Asia and has a large distribution in South America. In Brazil, it is popularly named as "colônia". The essential oil of *A. zerumbet* (EOAZ) has been reported by pre-clinical studies to exhibit antihypertensive activity, a pharmacological effect related to the presence of monoterpenes (1). A number of studies found this class of substances in EOAZ by gas chromatography (GC) analysis, mostly using capillary column coated with 5 % phenyl methyl-polysiloxane (2). However, a selective GC analytical method for the analysis of monoterpenes in EOAZ has not been reported. Therefore, the aim of this study was to develop a selective GC analytical method for analysis of monoterpenes in EOAZ using central composite design (CCD) combined with different column types. Fresh leaves of *A. zerumbet* were obtained from Plataforma Agroecológica de Fitomedicamentos (PAF) at Fundação Oswaldo Cruz, in the city of Rio de Janeiro, Brazil, in August 2014. The essential oil was extracted by hydrodistillation, using Clevenger apparatus. The CCD experiments were carried out, starting with a full factorial design (with a replicate at center point) with axial points. Three GC parameters were chosen as factors, oven initial temperature, heating rate and flow rate, beginning respectively with 70 °C, 4°C min^{-1} and 2 mL min^{-1} as central point, with a total of 16 runs and one replicate. The columns chosen were DB-5 (5% phenyl methylpolysiloxane), DB-1 (methylpolysiloxane), DB-17ht (50 % phenyl methylpolysiloxane), DB-35 (35 % phenyl methylpolysiloxane), DB-1301 (6 % cyanopropylphenyl methylpolysiloxane) and DB-1701 (14 % cyanopropylphenyl methylpolysiloxane). The method was developed in a CG/FID and the substances were identified by CG/MS. Helium was used as carrier gas and the oven final temperature was 290 °C. Chemical analysis revealed the presence of at least 36 components. Terpinen-4-ol, 1,8-cineole, sabinene and γ-terpinene corresponded to 22 %, 21 %, 20 % and 11 % of the crude oil, respectively. DB-17ht and DB-1, the higher and the lesser polar columns respectively, resulted in asymmetric and tailed peaks with poor separations. Intermediate polarity columns showed better results. Nevertheless, the best conditions were observed in DB-5 and DB-35 columns, all the peaks corresponding to the monoterpenes in DB-5 analysis had baseline resolution with exception of the separation between 1,8-cineole and limonene. However, a resolution of 1.56 was possible in DB-35, after optimization by central composite design. So, the final optimized method had an initial oven temperature of 70 °C, heating rate of 4°C min^{-1} and flow rate of 2 mL min with a DB-35 column. This is the optimized method ideally suited for the separation and identification of monoterpenes in EOAZ by gas chromatography.

1. Santos, B.A.,*et al*. J. Phytother.Phytopharm. 2011, **18**, 539-543.
2. Victório, C.P.; Leitão, S.G.; Lage, C.L.S. J. Essent.Oil Res., 2010, **22**, 52-54