DIRECT QUANTITATIVE ANALYSIS OF LINAMARIN IN CASSAVA BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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Cassava, *Manihot esculenta* Crantz, is a major source of dietary carbohydrate for human in many tropical countries. Roots and leaves of cassava are reported to contain cyanogenic glycosides: linamarin and to a lesser extent lotaustralin. Cyanide released from linamarin through the hydrolytic action of linamarase is generally accepted as the toxic agent responsible for numerous cases of poisoning [1].

Several methods for analysis of linamarin have been reported but all of them are based on the release of HCN from cyanogenic glycosides upon enzymatic hydrolysis [2]. We only found one report on the TLC semiquantitative determination of linamarin based on the visual estimation of fluorescence produced by a reaction with p-anisaldehyde [3].
This is why we have developed a simple, rapid and accurate quantitative procedure for direct determination of the intact glycoside.
We tried several mobile phases and post-derivatization systems based on the literature. It was necessary to use a double migration with two different mobile phases to separate linamarin from an interfering component. Aniline-diphenylamine-phosphoric acid [4] appeared to be the most specific reagent for the detection.

The following method was developed: a cassava sample was extracted by boiling 80% v/v methanol. Extracts and linamarin were deposited, in bands of 4 mm, on precoated silica gel $60F_{254}$ HPTLC plates (Merck), prewashed by development with methanol. The plates were first developed to a distance of 30 mm with the following system: ethylacetate-acetone-water (4:5:1, v/v/v), then to a distance of 85 mm using ethylacetate-formic acid-water (6:1:1, v/v/v) as the mobile phase. After each development, the plates were dried in a stream of warm air. The spots on the plates were vizualised by dipping with diphenylamine 2%, aniline 2%, phosphoric acid 15% in acetone, followed by heating at 105°C during 60 min. Densitometric quantification was effected at 525 nm by transmission scanning. The method was validated for accuracy, intra-day and inter-day reproducibility of peak area, linearity, and detection limit.
This HPTLC method was found to be very useful for the assay of linamarin in cassava-based meal.

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