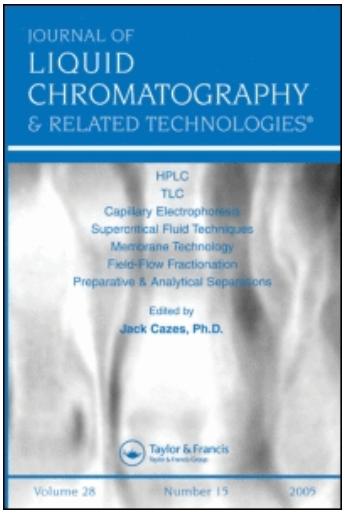

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Quantification of Usnic Acid in Usnea Florida by Densitometric High Performance thin Layer Chromatography

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QUANTIFICATION OF USNIC ACID IN *Usnea florida* BY DENSITOMETRIC HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

A densitometric HPTLC method was developed for the direct quantification of usnic acid in the lichen *Usnea florida*. The method involved a separation of usnic acid on a HPTLC plate in an appropriate eluent and *in situ* scanning of samples and standard zones. The percent recoveries for added usnic acid to samples were 102.75 to 106.29 % respectively. Both regression lines and correlation coefficients were used in calculation of usnic acid concentration in chloroform extract and in recovery rates.

INTRODUCTION

Usnic acid has been shown to possess important antibiotic and antineoplastic properties (1, 2) and hence is used for topical application in many pharmaceutical preparations (3). As usnic acid is a major component of various lichen species , the industrial application of

lichens, for example in animal feedstuff should be important. Thus it is necessary to find a rapid and accurate quantification method for usnic acid in these lichens.

The methods previously described for quantification of usnic acid in pharmaceutical preparations were mainly spectrophotometric and fluorometric methods (3). Both of these methods involved several steps of extraction, isolation and purification of usnic acid before its quantification. These methods certainly have disadvantage since they are time consuming and inaccurate.

This paper reported the direct quantification of the crude chloroform extract by densitometric HPTLC. The use of UV at 285 nm scanning is more rapid and more accurate than the use of chemical visualization (4).

MATERIAL AND METHODS

Lichen material

Usnea florida(L.)Wigg was collected in March 1989 in Trás-Os-Montes region of Portugal.

Standard Solutions

Usnic acid standard (U 023) was purchased from Tokio-Kasey.

Standard solutions were prepared at the concentrations of 1.4, 2.8, 4.2, 5.6, 7.0 and 14.0 μg/ml in chloroform.

Preparation of samples

Several samples of lichen *Usnea florida* (Table 1), were extracted with 100 ml of $CHCl_3$. After filtration, the chloroform solutions were

TABLE 1
Usnic acid content on *Usnea florida*

Samples	Weight (g)	g %
1	1.7074	4.11
2	1.7065	4.11
3	1.6940	4.01
4	1.4325	4.21
5	1.2912	3.77
6	1.0058	4.10
7	1.0055	3.58

diluted to an appropriate concentration. Each crude extract was submitted to HPTLC analysis together with usnic acid standard solutions.

HPTLC determination

HPTLC was carried out on Merck Plates (Art. nº 5641) in a Camag--Linear 28520 Chamber. The 2µl spots were applied with a Camag applicator. The plates were developed by toluene-glacial acetic acid (9:1).

Chromatograms of the crude extract mixed with a standard usnic acid did not show any significant difference due to migration distance.

Quantification of usnic acid (Table 1) was carried out by densitometry (Desaga-CD 60 Densitometer) in a UV region (285 nm). The method of quantification was based on the Kubelka-Munk's equation (5, 6). The detection limit of usnic acid by this method was shown to be about 1.4µg/ml by the calibration curves (Figure 1). Table 2 shows the result of a statistical treatment of the data.

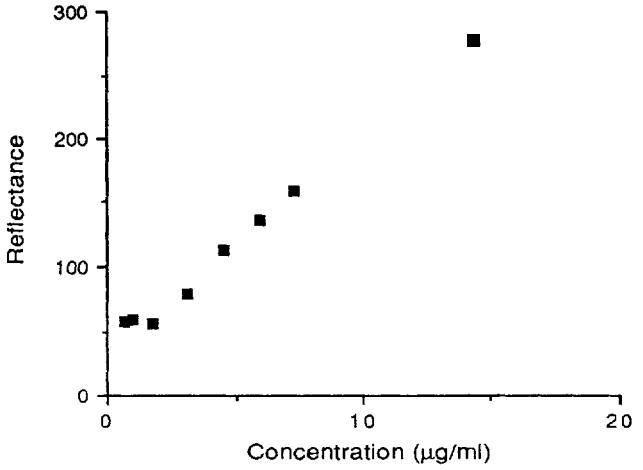

FIGURE 1
Detection limit on UV method

TABLE 2
Statistical data

Count	Mean (g%)	Confidence Interval (90%)
7	3.984	3.984 ± 0.166

TABLE 3
Recovery rates

Samples	mgUA/g lichen (mean value)	UA added (mg)	Recovered UA (mg)	Recovery(%)
R_1	39.84	39.10	41.77	106.29
R_2	39.84	85.70	88.27	102.75

UA = Usnic Acid

RESULTS AND DISCUSSION

In order to verify the extractive procedure, the recovery rates (R) were determined by addition of the usnic acid standard to the samples. The results of the recovery rates are shown in Table 3. These results showed that chloroform was an efficient solvent for extraction.

Peak areas were used as a parameter for calibration curve establishment with the suitable standard solutions previously referred.

Regression lines and correlation coefficients for chloroform extract and recovery rates determination were:

$y = 77.07 + 4.61x$; $r = 1.00$

$y = 125.44 + 7.60x$; $r = 0.99$

where

y = ordinate value

x = abscissa value

r = correlation coefficient

In the same manner, the formula used for calculation of usnic acid concentration was:

$Z = x/w$

where

Z = usnic acid content in g/100 g of sample

x = value of abscissa from regression line

w = weight of sample in g

On the recovery rates determination, the standard addition of usnic acid should be carefully done in order that the final concentration of usnic acid on the chromatographic plate must not exceed the range of calibration curve. Moreover, the amount of usnic

acid added should not provoke a "hidden effect" in the dilution procedure.

In conclusion, the HPTLC-densitometric UV scanning method has an advantage of being rapid, accurate and sensitive technique for quantification of usnic acid not only in lichens but also in pharmaceutical formulas.

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