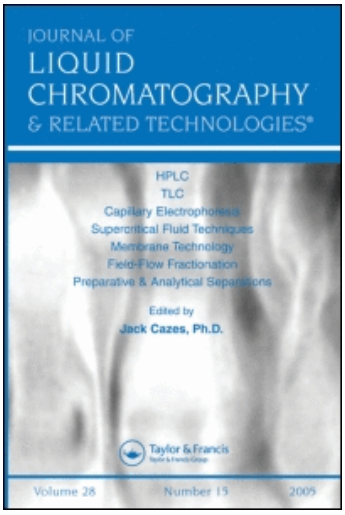

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HPLC ANALYSIS OF BOLDINE IN TABLETS AND SYRUP

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ABSTRACT

A rapid and reliable HPLC method, which allows the quantitative determination of Boldine in tablets and in syrup, is reported. The extraction of Boldine from powdered tablets was realized with alkalinized acetonitrile and the clear surnatant was directly injected. The extraction from syrup was realized with ethilic ether after sample dilution with water and saturation with sodium hydrogencarbonate; the organic layer was separated by centrifugation, dryed under nitrogen stream and the residue, collected with alkalinized acetonitrile, was directly injected. The chromatographic separation was achieved on a PRP - 1 10 μm HPLC column in isocratic conditions using a solvent mixture at pH 9.0 composed of water:acetonitrile:methanol:1M thrietilammonium acetate solution (50:25:25:5). A diode array detector was used at 304 nm with a reference wavelenght of 420 nm to minimize the possible interferences from the other components of the sample matrix.

INTRODUCTION

Boldine is an alkaloid held in Peumus Boldus leaves active in increasing biliary secretion, that has a local anestethic activity and an antispastic action on gastroenteric canal.[1,2] It is used in little hepatic insufficiences, biliary lithiasis, hepatic congestion states and cronic hepatites as an ingredient in choleretics and laxatives. For Boldine assay in pharmaceuticals only a densitometric assay was reported[3] and then a HPLC method seemed the more suitable for quantitation in tablets and syrup. In either two formulations together with Boldine are present other three active components: Cascara Sagrada dry extract, Rhubarb dry extract and α - 1-hydroxy-cycloesilbutirric acid.
Different extraction methods were developed for the two formulations but the sample solutions were analyzed in the same chromatographic conditions on a reversed phase HPLC system.

EXPERIMENTAL

Equipment: The liquid chromatograph used was an Hewlett-Packard 1090L equipped with autoinjector, autosampler, diode array UV detector HP 1090 M series II connected to a work station with Hard Disk, printer and plotter.

HPLC conditions: A polymeric reverse phase LC-column (PRP - 1 10 µm 250 x 4.1 mm, Hamilton Co, Reno, NV) with an identic guard column was used. The mobile phase for isocratic elution was a mixture of water, methanol, acetonitrile and 1 M triethilammonium acetate solution at pH 7.0 (50/25/25/5 v/v/v/v); the mixture pH value was adjusted at 9.0 with a 20 % sodium hydroxide solution. The flow rate was set at 0.7 ml/min, the autoinjector at 10 µl and the oven temperature at 40 °C. The detector was set at 282 nm with a bandwidth of 4 nm and as reference wavelenght was selected 420 nm with a bandwidth of 8

nm. In these operative conditions the Boldine peak showed a retention time of about 10.6 minutes.

Materials and Reagents: Water (Waters Purification System Milli Q), methanol and acetonitrile (LiChrosolv, Merck, Darmstadt, Germany) were HPLC grade. Ethilic ether, triethilammine, glacial acetic acid, ammonium hydroxide 30 % (RPE, Carlo Erba, Milan, Italy), acetonitrile, sodium hydrogen carbonate (PA, Merck) were reagent grade and were used as received. Reference Boldine were obtained from Indena S.p.A (Milan, Italy). Neo Menabil Complex tablets each containing 0.5 mg of Boldine and Neo Menabil Complex syrup containing 3.4 mg of Boldine for each 100 g , were manufactured by Menarini Industrie Farmaceutiche Riunite s.r.l. (Florence, Italy).

Reference Solution: A stock reference solution of Boldine was prepared by accurately weighing 20 mg of reference Boldine into a 20 mL volumetric flask and dissolving it with acetonitrile. For analysis a working solution was prepared by diluting 5.0 mL of stock solution to 100 mL with acetonitrile. This working solution was freshly prepared while the stock one was stable at room temperature at least a week.

Sample Solutions:

a) Tablets: each tablet was grinded in a mortar and the correspondent powder, accurately weighed, was transferred in a Sovirel vial and added with 10.0 mL of acetonitrile containing 0.1 % of 30 % ammonium hydroxide solution. The sample was sonicated for 15 minutes and the resulting sospension centrifuged at 2500 rpm for 5 minutes. The clear supernatant was directly injected onto LC-column.

b) Syrup: about 2 g of syrup, exactly weighed, was diluted with 1 mL of water and added with sodium hydrogen carbonate till saturation. The mixture was extracted with 4.0 mL of ethilic ether shaking for 10 minutes and

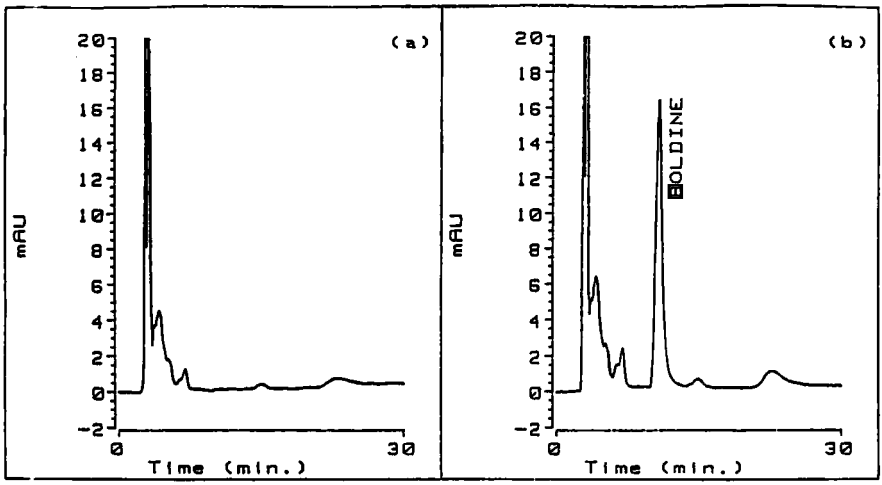

Fig. 1: (a) Chromatographic profile of blank tablet.
(b) Chromatographic profile of tablet sample.

centrifuged at 2500 rpm for 5 minutes; 3.0 mL of the organic layer was collected in a conical vial and evaporated to dryness under nitrogen. The residue was collected with 1.0 mL of acetonitrile containing 0.1 % of 30 % ammonium hydroxide solution and direcly injected onto LC column.

RESULTS AND DISCUSSION

Under the selected conditions syrup and tablets solutions give rise to the HPLC chromatograms reported in fig. 1 and fig. 2 respectively. In both cases the peak at the same RT of Boldine reference solution chromatogram had the same UV spectrum and, moreover,they resulted pure by analyzing the UV spectra collected at different times during eluition of chromatographic peak (peak purity

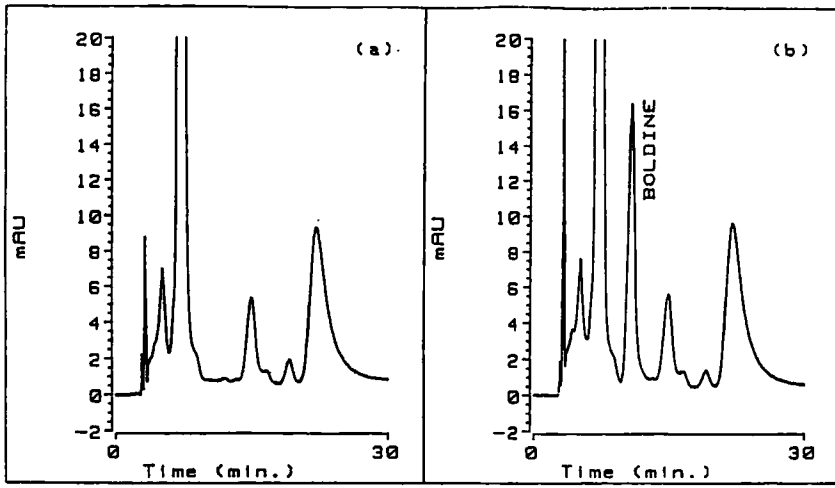

Fig. 2: (a) Chromatographic profile of blank syrup.
(b) Chromatographic profile of syrup sample.

calculated by Hewlett-Packard software was > 99). The specifity of the chromatographic analysis was tested by analyzing either for tablets or syrup Boldine free mixture of the formulations components and the relative chromatograms are shown respectively in fig 1 and 2 together the correspondent sample chromatogram.

Solutions of Boldine reference substance were prepared in the range 25-100 µg/mL and calibration data obtained by HPLC under the described chromatographic conditions were statistically analyzed. Linear regression analysis of peak area data yielded a slope of 80.701, an intercept of 22.14 and a coefficient of determination of 0.999. Chromatographic reproducibility, expressed as coefficient of variation calculated by injecting 6 replicates working solution of reference Boldine, was 0.14 %. The accuracy for tablets Boldine assay was calculated by analyzing for 6 times a free Boldine mixture of the tablets components spiked with a known amount of Boldine (500 µg for

TABLE 1

Accuracy and precison for Boldine assay in tablets and syrup.

	Accuracy	Precison
Tablets	100.46 ± 0.88	1.09 %
Syrup	99.45 ± 1.78	1.47 %

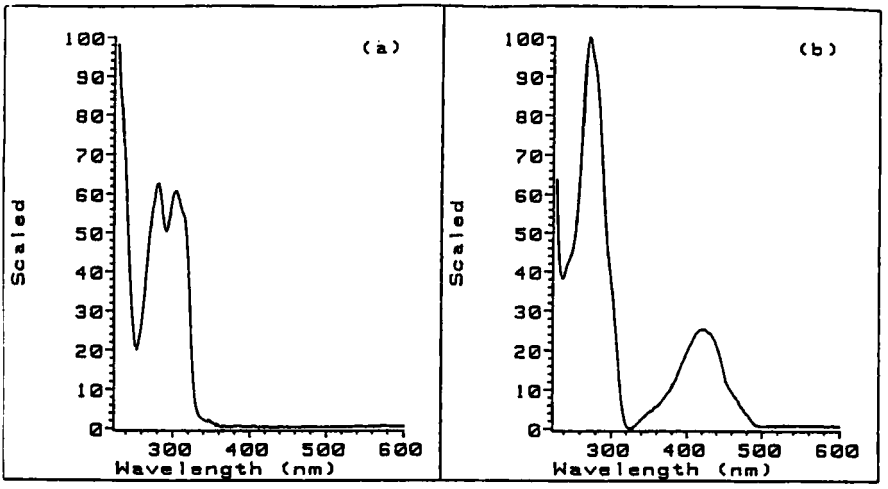

Fig. 3: (a) UV spectrum of Boldine.
(b) Typical UV spectrum of interfering components of Rhubarb dry extract.

theoretical tabler weight) by the proposed analytical procedure. The found amounts of Boldine were compared with the actual ones and the accuracy was expressed as percentage of the recovery. The accuracy of the method for syrup Boldine assay was calculated by analyzing 6 samples of Boldine free syrup spiked with known amounts of Boldine (65 μg for 2 g); the found values were comparised with the actual ones and the accuracy was expressed as percentage of the recovery. The data showed that quantitative recovery of Boldine was obtained in either two cases and that the method was accurate (the 100% value of recovery is included between the confidence limits of the average value) and precise (the precision is given from the relative standard deviation of recovery values).

The minimum quantifiable level (MQL) for Boldine assay in tablets was set at 2.5 μg/mL (corresponding to 7.2 % of the theoretical potency for tablets and 5.1 % for syrup. MQL was calculated by multiplying for 10 times the ratio between the standard deviation value (calculated for 4 replicates of the reference Boldine solution at the lowest concentration) and the sensitivity (expressed as the the value of the slope of the calibration curve).

The diode array detector, used in this method, allow to maximize the detector response chosing a reference wavelenght value that allow to minimize the interference of other componentes of the tablet and syrup. The best value of analytical wavelenght was 304 nm with a bandwidht of 4 nm and 420 nm with a bandwidht of 8 nm for reference wavelenght. This choice allows to obtain a very clean chromatographic profile because the possible interfering peaks coming from Rabarbaro dry extract that have an UV spectrum reported in fig.3 together with the Boldine one, are, in these acquisition conditions, completely suppressed because the absorbance value at 304 nm is the same that at 420 nm, while Boldine practically does not absorbe at the reference wavelenght.

The proposed HPLC method have the advantage that the preparation step of the samples is very short and simple

and its precision, accuracy and specificity allow to obtain reliable results in short time.

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