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Differential pulse polarographic determination of total benzophenantridinium alkaloids in *Sanguinaria* extract-based oral rinses

S. Pinzauti, E. La Porta, F.F. Vincieri, P. Gratteri, G. Papeschi and M. Bambagiotti-Alberti

Department of Pharmaceutical Sciences, University of Florence, Florence (Italy)

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Summary

A differential pulse polarographic method was developed for the determination of total benzophenantridinium alkaloids in *Sanguinaria canadensis* extract-based oral rinses without prior extraction procedures, McIlvaine buffer (pH 3.4) being employed as the supporting electrolyte. The electrochemical reduction involves the transfer of one electron and potential of the single peak occurs between −0.39 and −0.44 V vs Ag/AgCl as a function of the different relative percentage of benzophenantridinium alkaloids. The alkaloid amount was expressed as chelerythrine chloride and calculated by means of a calibration graph. A column chromatographic procedure to obtain chelerythrine chloride reference standard is described. Common oral rinse excipients and zinc ions were found not to interfere.

Introduction

The extract from the rhizome of *Sanguinaria canadensis* L. *(Sanguinaria)*, contains a significant amount of mixed benzophenantridinium alkaloids, predominantly sanguinarine (**I**) and chelerythrine (**II**) chlorides (Boulware et al., 1985) whose pharmacological activity was found to be similar to that of chelidonine (**III**), the principal benzophenantridine alkaloid of *Chelidonium majus* L. (Benigni et al., 1964). *Sanguinaria* extract has been used in homeopathic and folk medicines in North America for more than 100 years (Etemadzadeh and Ainamo, 1987); recently the anti-plaque effectiveness of sanguinarine has been reported to be due to its antibacterial action (Dzink and Socransky, 1985) and to the specific sorption and retention in the oral cavity, both in plaque and saliva, following a standard regimen of a *Sanguinaria* extract oral rinse (Southard et al., 1984). Although the results of subsequent clinical trials on the plaque-inhibitory effect of sanguinaria extract mouth-rinses are conflicting (Wennström and Lindhe, 1985; Parsons et al., 1987; Etemadzadeh and Ainamo, 1987; Afseth and Rölla, 1987), there is a certain interest in *Sanguinaria* extract as the claimed active ingredient of some

Correspondence: S. Pinzauti, Università di Firenze, Dipartimento di Scienze Farmaceutiche, Via G. Capponi 9, 50121 Firenze, Italy.

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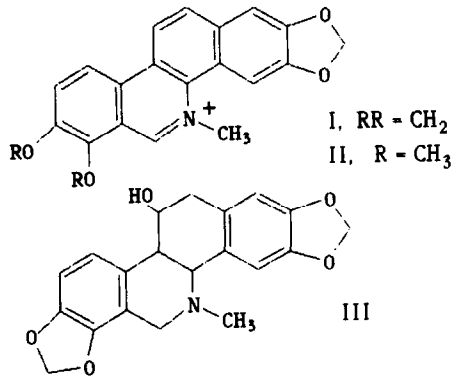

Formulae I–III.

pharmaceutical preparations marketed for the prevention or reduction of caries and periodontal diseases.

The sum of sanguinarine and chelerythrine in the plant rhizome was found to be about 70% of the total alkaloid content (Slavik and Slavikova, 1960). Recently the relative percentages of benzophenantridinium alkaloids in a *Sanguinaria* extract have been evaluated, the reported values for sanguinarine and chelerythrine being 50% and 25%, respectively (Thorne et al., 1986).

Besides, the antimicrobial activity of chelerythrine was found to be similar to that of sanguinarine (Mitscher et al., 1978), so that the total benzophenantridinium alkaloid content, consisting mainly of sanguinarine and chelerythrine, could be responsible for the anti-plaque effectiveness of *Sanguinaria* extract mouthwashes. Therefore, the search for a simple and reliable method for assaying total benzophenantridinium alkaloids appears to be of interest and has been the aim of this work.

Some spectrophotometric methods following extraction and thin-layer chromatography have been described for determining chelerythrine and/or sanguinarine (Maslova, 1974; Scholz et al., 1976; Balderstone and Dyke, 1977; Shenolikar et al., 1981), but the only specific procedure for pharmaceutical preparations has been an HPLC analysis of sanguinarine (Thorne et al., 1986).

Since the polarographic behaviour of benzophenantridinium alkaloids has not been reported, we describe here the differential pulse polarographic (d.p.p.) reduction of these drugs at the dropping mercury electrode and the optimum conditions for their quantitation in oral rinse dosage form.

Materials and Methods

Reagents and chemicals

Sanguinaria fluid extract (60% ethanol, from Lifepharma, Milan, Italy), chelidonine (Sarget, Mérignac, France), impure sanguinarine nitrate and chelerythrine chloride (Roth, Karlsruhe, F.R.G.) and polysorbate 80 (Merck-Schuchardt) were used. Samples of two brands (A and B) of *Sanguinaria* extract mouth-rinse were purchased in pharmacies. Their claimed percent (w/v) composition was as follows:

Brand A: *Sanguinaria* fluid extract (from the above supplier) 3; sodium monofluorophosphate, 0.18; xylitol, 7.5;

Brand B: *Sanguinaria* extract, 0.03; zinc chloride, 0.2; alcohol, 10; citric acid, 0.03; sodium saccharin, 0.10; polysorbate 80, 0.6; poloxamer 407, 0.1.

All other chemicals, including water, were analytical reagent grade. McIlvaine buffer (pH 3.4) was made by mixing 28.5 ml of 0.2 M disodium hydrogen phosphate and 71.5 ml of 0.1 M citric acid.

Apparatus

A Metrohm E 506 recording polarograph equipped with a Metrohm VA 663 stand and multi-mode electrode assembly was used. Polarography was performed in the differential pulse mode with a -50 mV pulse being applied using a 1 s forced drop time and a scan-rate of 4 mV/s. A 3-electrode operation was employed with a pneumatic control dropping mercury electrode, a glassy carbon auxiliary electrode and a silver/silver chloride (3 M KCl) reference electrode. Scans were performed from 0 to -0.7 V vs Ag/AgCl with a full scan range of 2.0 V. The usual current sensitivity required was 0.15–0.25 μA for a peak response of full-scale deflection. For ascertaining the effects of the corrected mercury pressure on the peak height (the mercury column ranged from

50 to 75 cm), a traditional polarography stand (Metrohm E 505) was used.

The number of electrons involved in the reduction process was calculated at a chelerythrine chloride concentration of 2.5×10^{-4} M using a PAR 174A solid-state controlled-potential coulometric apparatus. The experiment was carried out in the absence of air in a 5 ml cell with a mercury pool as the working electrode, a platinum coil as auxiliary electrode and a fixed potential of -0.44 V vs Ag/AgCl.

Preparation of chelerythrine chloride reference standard

500 mg of chelerythrine chloride were chromatographed on a silica gel column (E. Merck, 70-230 mesh ASTM, 50×2 cm i.d.) using toluene–methanol (9.5 : 0.5) as eluent to yield seventy 3-ml fractions which were each chromatographed by HPTLC (E. Merck, silica gel 60) in toluene–methanol (9 : 1) to verify their purity. Two pools were evaluated (1–52 and 53–60 fractions). Concentration under reduced pressure ($T < 30\,^\circ$C) gave a residue that was heated with acetone, filtered and dried; yield 20%; m.p. 270 °C (decomp.). The purity of the obtained chelerythrine chloride was monitored by HPTLC (toluene–methanol 9 : 1) and confirmed by elemental analysis, UV (Hruban et al., 1970) and ^{1}H-NMR data.

The same chromatographic process can be used to purify commercial samples of sanguinarine salts (with lower yield).

Calibration graph

Pure chelerythrine chloride stock solution was prepared by dissolving 25 mg of the drug in 15 ml of ethanol and 1 ml of 1 M hydrochloric acid in a 25-ml volumetric flask and diluting to volume with ethanol after stirring for 2 h at 50 °C.

A calibration graph was prepared by introducing into the polarographic cell 15 ml of a blank solution (10 ml ethanol, 0.6 g polysorbate 80 and 10 ml of McIlvaine buffer, pH 3.4, made to 100 ml with water), 0.05 ml of 1-octanol (as an anti-foaming agent) and a sequence of appropriate aliquots (with micropipettes) of chelerythrine chloride stock solution to achieve final concentrations of 10–50 μg/ml. After deaeration for 10 min the polarograms were recorded at 25 °C using the previously described parameters. The resulting current was calculated on the basis of peak heights measured with reference to control solution polarogram (Fig. 1). Samples of 12 concentrations were run 4 times and gave a correlation coefficient for the graph of 0.9982.

Analysis of sanguinaria fluid extract or oral rinses

1.5 g of *Sanguinaria* fluid extract were weighed in a 50-ml calibrated flask, added to 5 ml of ethanol and 0.3 g of polysorbate 80 and diluted to volume with water. 10 ml of this solution (or 10 ml of commercial oral rinse), 10 ml of McIlvaine buffer and 0.05 ml of 1-octanol were introduced into a 100-ml calibrated flask and diluted to volume with water to obtain the assaying sample, 15 ml of which were introduced into the polarographic cell. The solution was purged for 10 min with nitrogen prior to recording the polarogram as above. The concentration of total benzophenantridinium alkaloids expressed as chelerythrine chloride was calculated by the calibration graph.

Results and Discussion

Commercially available sanguinarine or chelerythrine samples were actually a mixture of benzophenantridinium alkaloids; on the other hand, because of its higher chromatographic purity, the above-described purification procedure was more conveniently applied to chelerythrine, obtaining a chelerythrine chloride authentic sample that was used as reference standard throughout this work.

Six benzophenantridinium alkaloids were detected in *Sanguinaria* extract, viz. sanguinarine, chelerythrine (the two principal components), sanguilutine, chelirubine, sanguirubine and chelilutine. The relative average molecular weight, taking into account their relative percentage (Thorne et al., 1986), was calculated to be 349.6. Therefore our quantitation of total benzophenantridinium alkaloid content expressed as chelerythrine (mol. wt. = 348.4) was a good approximation of the real amount present in sanguinaria extract-based oral rinses.

An acidic pH range was found to be required for benzophenantridinium alkaloids (Slavik and Slavikova, 1960) or *Sanguinaria* extract (Thorne et al., 1986) stability in aqueous solutions. To obtain controlled pH values, a McIlvaine buffer was selected as supporting electrolyte over the pH range 2.6–4.0. In these conditions the differential pulse polarography provided a single, well resolved peak (Figs. 1 and 2) suitable for precise and accurate determination of benzophenantridinium alkaloid content in oral rinse dosage form. The peak occurred at $E_p = -0.392$ V and -0.440 V vs Ag/AgCl for sanguinarine and chelerythrine, respectively. It should be noted that although sanguinarine and chelerythrine can be distinguished when examined individually, their quantitative resolution was not possible when both compounds were present in a mixture. Thus, the observed E_p of *Sanguinaria* fluid extract and commercial oral rinses occurred at intermediate values between the two above mentioned (Fig. 2).

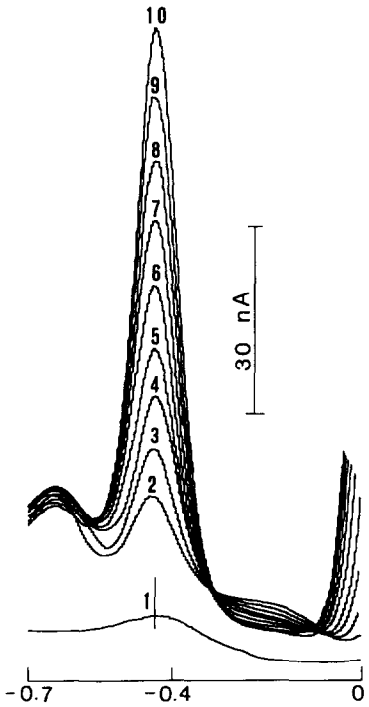

Fig. 1. Variation of d.p.p. peak height with chelerythrine chloride concentration: (1) 0, (2) 12.9, (3) 14.5, (4) 16.0, (5) 17.6, (6) 19.2, (7) 20.8, (8) 22.3, (9) 23.9, (10) 25.5 μg/ml. Abscissa: volts vs Ag/AgCl.

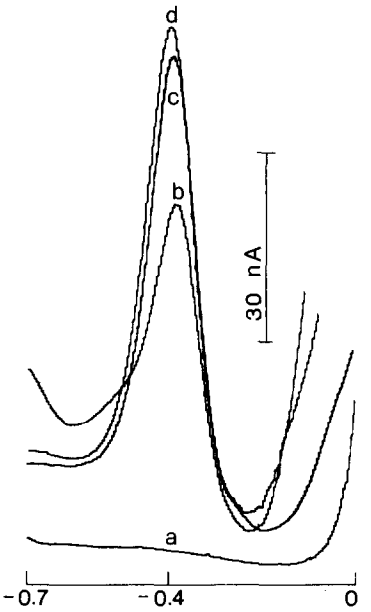

Fig. 2. D.p.p. peaks of *Sanguinaria* extract-based commercial products: (a) control solution, (b) Brand B oral rinse, (c) 3% *Sanguinaria* fluid extract, (d) Brand A oral rinse. Abscissa: volts vs Ag/AgCl.

The peak current varied linearly with the concentration of chelerythrine chloride over the assayed range of 3.3×10^{-5} to 1.3×10^{-4} M with an average recovery (8 determinations) at the 25 μg/ml level of $99.3 \pm 1.4\%$. Hence, using the described experimental parameters, the method is sensitive to about 12 μg/ml, which is well below the necessary concentration for dosage form assay. It should be noted that the calibration graph showed a non-zero intercept and therefore it is recommended that for highest accuracy the concentration–diffusion current plot method be used instead of the standard addition technique.

The results of d.p.p. analysis of total benzophenantridinium alkaloid content of a *Sanguinaria* fluid extract and two brands of oral rinses are presented in Table 1. Manufacturer data of Brand A alkaloid content were not available, but the obtained result was consistent with the labelled 3% *Sanguinaria* fluid extract (Fig. 2c, d). A good agreement was noted with the manufacturer's literature of Brand B claiming the presence of 0.010% sanguinarine chloride (Boulware et al., 1984), considering that the relative amount of

TABLE 1

D.p.p. analysis of total benzophenantridinium alkaloid (t.b.a.) content of two Sanguinaria extract-based oral rinses and a Sanguinaria fluid extract

t.b.a. content expressed as chelerythrine chloride.

Product	*t.b.a. content found* [a] *% w/v (± S.D., n = 4)*	label claim, % w/v
Brand A	0.0239 (0.0003)	3% *Sanguinaria* fluid extract
Brand B	0.0193 (0.0002)	0.030% *Sanguinaria* extract [b]
Sang. fl. extr.	0.767 (0.010)	–

[a] Expressed as chelerythrine chloride.
[b] Dried extract equivalent to 0.010% sanguinarine chloride (Boulware et al., 1984).

sanguinarine in *Sanguinaria* extract was found to be 50% of total benzophenantridinium alkaloids (Thorne et al., 1986).

The d.p.p. peak is attributable to the reduction of the imminium group of benzophenantridinium alkaloids; in fact, the parent benzophenantridine alkaloid chelidonine (**III**) was not polarographically reducible. Coulometric analysis indicated that one electron per molecule was transferred in the electrochemical process. The polarographic behaviour of benzophenantridinium alkaloids at the dropping mercury electrode was found to be consistent with the one-electron electrodimerization described for the reduction of imminium cations (Andrieux and Savéant, 1968; 1970).

The peak current was independent of pH in the studied range 2.6–4.0 and unaffected by changes of buffer concentration. These observations and the constancy of the product $i_d \cdot h^{-1/2}$, with a low temperature coefficient, implied that the current was controlled essentially by diffusion. The reversibility of the electrode process was assessed in several ways: peak potential did not vary with drop time or temperature, the peak height in forward and reverse scan was equal and, above all, the peak half-width was very close to the theoretical value of 90.4/n mV at 25 °C (Bond, 1980).

The d.p.p. method has the potential for application to shelf-life testing in those instances where the decomposition process involves cleavage of the imminium group, as the electrochemical process occurs specifically at that moiety. Common oral rinse excipients and other ingredients, comprising zinc ions (present in Brand B), did not interfere in this simple, sensitive and rapid method which was performed with a low-cost apparatus and without prior extraction procedures.

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