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CO-EXTRACTION-SPECTROPHOTOMETRIC DETERMINATION OF BERBERINE WITH QUININE AND BROMOPHENOL BLUE

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A sensitive and selective method for the determination of berberine is described. Though only a little berberine is extracted with bromophenol blue into chloroform at pH 6.7, it is extracted in quantity with the dye when quinine, being a bulky tertiary amine, co-exists in the aqueous solution. The blue associate is used for selective spectrophotometric determination of berberine in multi-component drugs.

As dyes such as bromophenol blue (BPB),[1] bromocresol green (BCG)[2] and bromothymol blue (BTB)[3] are diprotic acids, color reactions arising from the media buffered at varied pH values are complicated. For example, BPB reacts with both bulky amines and quaternary ammonium compounds in a pH 3.8 medium and forms yellow colored salts. The associates [$HR_3N^+ \cdot BPB^-$] are readily extracted from acidic aqueous solutions with organic solvents. On the other hand, in alkaline media only quaternary ammonium compounds form blue colored salts with BPB, never amines, and these ion-associates [$2R_4N^+ \cdot BPB^{2-}$] can also be extracted into organic solvents. Though useful methods can be developed for the determination of minute amounts of various amines and quaternary ammonium compounds in pure forms, the extraction of the associates is not selective in either media described above.

Tatsuzawa et al[4] reported the selective determination of quinine, in a pH 6.2 medium using BPB and solvent extraction, but the method is not sensitive.

In the present investigation we found that berberine, being a quaternary ammonium salt, was successfully co-extracted with BPB into chloroform in a pH 6.7 medium, but only in the presence of quinine. Extraction was not possible in the absence of quinine. Accordingly, berberine can be determined spectrophotometrically without disturbance of other quaternary ammonium compounds and amines.

This paper described the sensitive and selective determination of berberine in multi-component commercial samples.

Take a sample solution containing 0.2 - 1.2 μg ml^{-1} of berberine hydrochloride in a 50 ml volumetric-flask and 1 ml of 2.4 x 10^{-3}M BPB solution buffered at pH 6.7, 10 ml of phosphate-borate buffer solution (pH 6.7) and 1 ml of 5 x 10^{-4}M quinine standard solution. Dilute the mixture to the mark with distilled water and transfer the solution into a 100 ml separatory funnel and shake with 10 ml of chloroform for 5 min, then centrifuge to remove water droplets. After separation of the organic layer, measure the absorbance of the organic phase at 610 nm against a reagent blank.

Fig. 1 shows absorption spectra of ion-associates formed between onium compounds and the BPB anion. Both quinine and berberine were extracted into chloroform with BPB at pH 3.8 and both extracts showed a yellow color having a wavelength of 418 nm (curves 1 and 2). On the other hand, the absorption maximum of the quinine-BPB associate [$2HQui^{+} \cdot BPB^{2-}$] occurred at 590 nm in a pH 6.7 medium (curve 4), though the berberine was not entirely extracted with BPB in the same medium (curve 3). Other amines such as procaine, dibucaine and chlorpheniramine were not extracted, either. Therefore, the extraction with BPB at pH 6.7 is selective but not sensitive for the assay of quinine.

Of quaternary ammonium compounds, berberine and benzethonium were extracted at pH 6.7 only with the co-existence of 1 - 1.5 x 10^{-5}M quinine. The extractability of berberine was very high (curve 5). ΔE for 2 x 10^{-6}M berberine (Fig. 1) was 0.375, where $\Delta E = E_{mix} - E_{qui}$, E_{mix} represents the absorbance of the 1 x 10^{-5}M quinine berberine mixture, and E_{qui} is the absorbance of the quinine associate. The absorption maximum of the mixture was shifted to 610 nm.

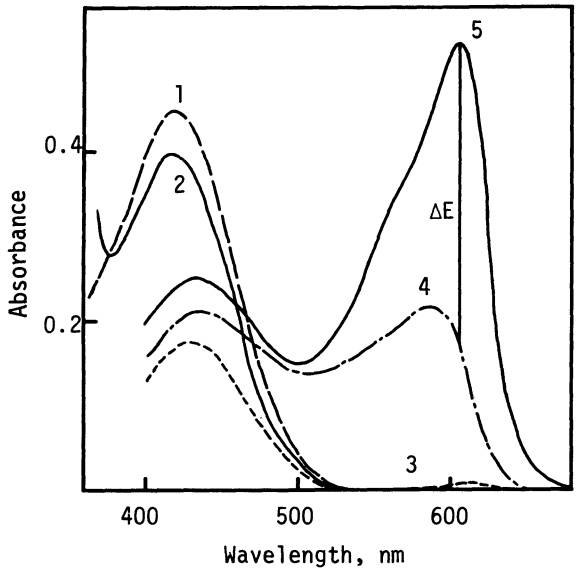


Fig. 1 Absorption spectra of BPB-Quinine, -Berberine and BPB-Quinine-Berberine associates at different pH
1: 3 x 10^{-6}M Quinine, 2: 4 x 10^{-6}M Berberine, pH on extraction: 3.8, λ_{max}: 418 nm
3: 2 x 10^{-6}M Berberine, λ_{max}: 610 nm
4: 1 x 10^{-5}M Quinine, λ_{max}: 590 nm
5: 2 x 10^{-6}M Berberine with 1 x 10^{-5}M Quinine, λ_{max}: 610 nm, pH on extraction: 6.7, BPB concentrations: 1,2: 3.2 x 10^{-4}M, 3,4,5: 4.8 x 10^{-5}M

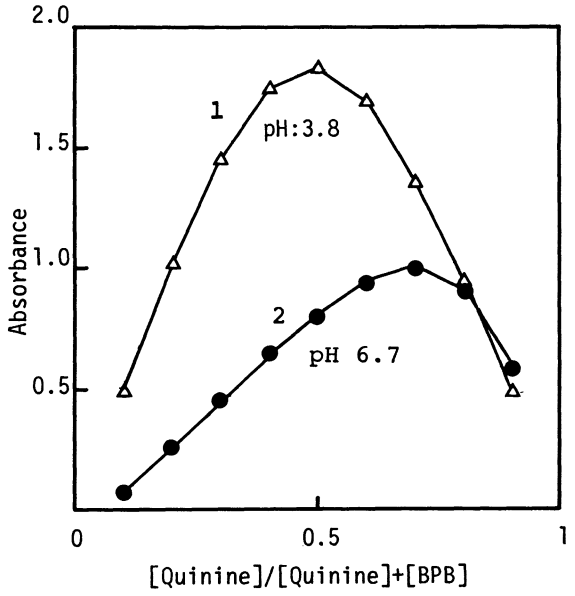
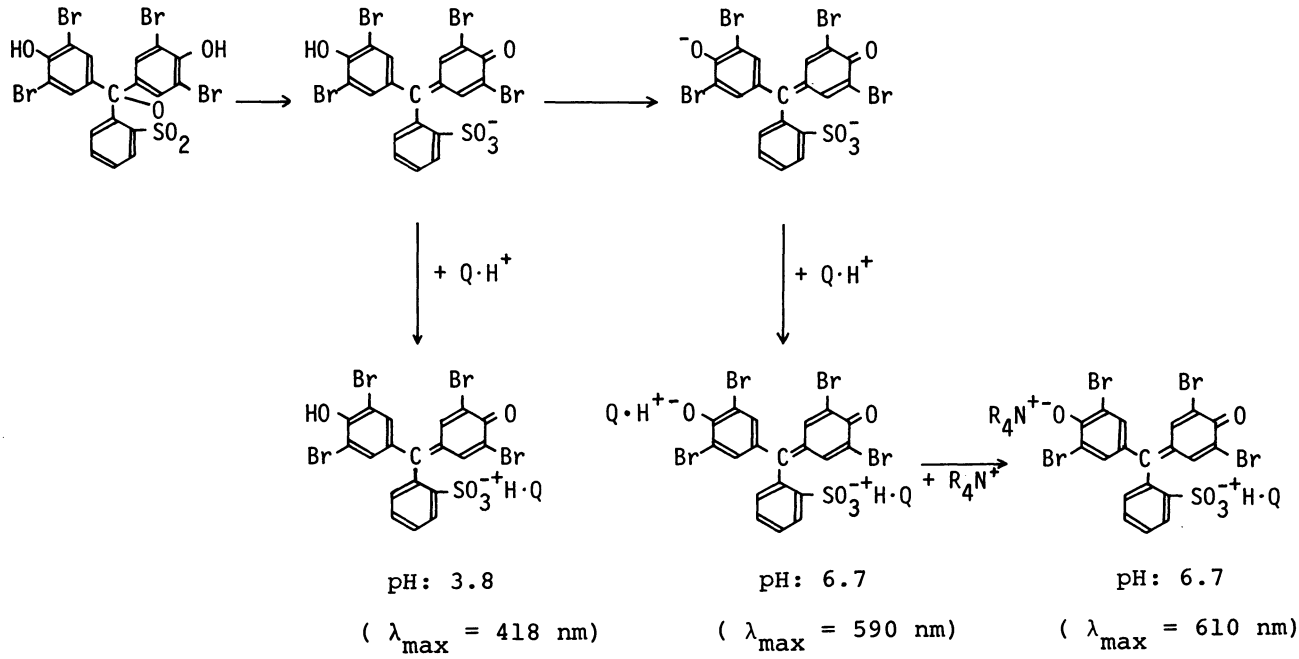


Fig. 2 Continuous variation plots of BPB-Quinine associates at different pH
[Quinine] + [BPB] = 4 x 10^{-5}M
Wavelength: 1 —△—△— 418 nm
2 —●—●— 590 nm
Solvent: Chloroform, Reference: Reagent blank

The following extraction scheme can be assumed by the continuous variation plots, shown in Fig. 2.



+HQ: Quinine cation, R_4N^+: Quaternary ammonium cation

Scheme I

The pH on the extraction of the BPB-quinine-berberine associate [BPB-Qui^-·Ber^+] was at a maximum in the pH 6.7 medium. The effect of BPB concentration on the color development was examined. It was observed that the extraction of berberine in the presence of 1 x 10^{-5}M quinine was complete and constant from 1.6 to 4.8 x 10^{-5}M BPB. The color intensity of the chloroform phase remained constant for at least 30 min at 25°. Other conditions for the determination of quinine with BPB are already established.[4,5]

To a solution containing 2 x 10^{-6}M berberine and 1 x 10^{-5}M quinine, various amounts of foreign compounds were added and their interferences examined by the procedure. The usual tablet diluents and co-additives which accompany berberine in

Table I Analysis of berberine in commercial samples by the proposed method

Components	Certified mg/Tablet	Found mg	Certified mg/Tablet	Found mg
guanoflacin	15		15	
acrinol	10		10	
berberine	30	31.2*	40	40.5*
chlorpheniramine	0.75		0.75	

* The values were the average for 3 measurements.

pharmaceuticals, such as glucose, lactose, starch, dibucaine and chlorpheniramine, were found not to interfere in the analysis. Other quaternary ammonium cations such as neostigmine, sparteine, tetraethylammonium and methylatropine did not interfere, either.

Table I shows the results for the commercial samples analyzed by the proposed method. Acrinol and chlorpheniramine, which gave some interference in a pH 3.8 or an alkaline medium,[6] did not interfere in this method.

Consequently, co-extraction spectrophotometry is available and simple for the selective determination of berberine in multi-component drugs.

References

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