Short report

Spectrophotometric determination of total alkaloids in some Iranian medicinal plants

Fazel Shamsa[1], Hamidreza Monsef[2], Rouhollah Ghamooshi[2] and Mohammadreza Verdian-rizi[2*]

[1]Department of Medicinal Chemistry, [2]Department of Pharmacognosy, Faculty of Pharmacy,
Tehran University of Medical Sciences, Tehran, Iran
**Corresponding author: Tel: + 98 21 44560215 Fax:+ 98 21 88784521*
E-mail address: mverdian@razi.tums.ac.ir

Abstract:

A simple spectrophotometric method based on the reaction with bromocresol green (BCG) has been developed for determination of total alkaloids in medicinal plants. A yellow complex forms and is easily extractable by chloroform at pH 4.7. The absorbance of the complex obeys Beer's law over the concentration range of 4-13 μg atropine per ml of chloroform. This procedure can be carried out in the presence of other compounds without interference.

Keywords: Atropine; Beer's law; Bromocresol green; Medicinal plants; Total alkaloids

Introduction

The alkaloids represent a group of natural products that has had a major impact throughout history on the economic, medical, political and social affairs of humans. Many of these agents have potent physiological effects on mammalian systems as well as other organisms, and as a consequence, some constitute important therapeutic agents. Atropine, morphine, quinine and vincristine are representative of a host of agents used to treat a range of disease conditions that range from malaria to cancer. Therefore determination of total alkaloids is very important related to the quality of medicinal plants [1].

The methods reported for the determination of alkaloids include official methods [2-3], high-performance liquid chromatography (HPLC) [4-7], fluorimetry [8-9], ion chromatography [10], coulometry [11], gas chromatography [12], and electro chromatography [13]. Most of the reported spectrophotometric methods suffer from disadvantages such as narrow range of determination. They require heating or extraction, a long time is needed for the reaction to be completed, and the colored product formed is unstable. The purpose of the current work was to provide a simple, sensitive, and rapid spectrophotometric method for the determination of total alkaloids in medicinal plants. The method is based on the reaction of alkaloid with bromocresol green (BCG), forming a yellow-colored product. The method offers the advantages of sensitivity and stability.

Materials and methods

Plant Material

Plant materials including *Acroptilon repen* L. (aerial parts), *Berberis vulgaris* L. (aerial parts, fruits), *Biebersteinia multifidia* DC. (aerial parts, root), *Calendula officinalis* (flower), *Chelidonium majus* L. (aerial parts), *Echium amoenum* Fish & Mey (flower), *Equisetum arvense* L. (aerial parts), *Hyoscyamus niger* L. (aerial parts), *Hypecoum pendulum* L. (aerial parts), *Malva sylvestris* L. (aerial parts), *Scrophularia striata* Bioss. (root) and *Stachys lavandulifolia* Vahl. (aerial parts), collected from local market of Tehran province, in May 2003. All plants were identified in the Herbaruim of Faculty of Pharmacy, Tehran University of Medical Sciences.

Preparation of solutions

Bromocresol green solution (1×10^{-4}) was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water). Atropine standard solution was made by dissolving 1 mg pure atropine (Sigma Chemical, USA) in 10 ml distilled water.

Preparation of standard curve

Accurately measure aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of atropine standard solution and transfer each to different separatory funnels. Then, add 5 ml pH 4.7 phosphate buffer and 5 ml BCG solution and shake a mixture with 1, 2, 3 and 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine.

Extraction

The plant materials (100g) were ground and then extracted with methanol for 24 h in a continuous extraction (soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45 °C to dryness. A part of this residue was dissolved in 2 N HCl and then filtered. One ml of this solution was transferred to a separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and the complex formed was extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking. The extracts were collected in a 10-ml volumetric flask and diluted to volume with chloroform.

The absorbance of the complex in chloroform was measured at 470 nm.

Results and discussion

A yellow-colored complex with a maximum absorption was developed. This complex was completely extractable by chloroform at pH 4.7. A calibration curve was plotted for various concentrations of atropine (Figure 1). Beer's law was followed over the concentration range of 4-13 µg atropine per mL of chloroform. The effects of temperature and pH were studied. A pH 4.7 gave optimum results and different temperatures had no effect on complex formation and extraction. The complex was very stable in chloroform and began to fade slowly only after 10 days. Before the extraction, the mixture was put in a boiling water bath for 3 min. The absorbance did not change after extraction with chloroform. The plant materials have been extracted by a method that only alkaloids come into the final residue and therefore other organic compounds which react with BCG, do not exist in the final solution [14]. Table 1 shows the amount of total alkaloid in tested plant materials determined by BCG-complex formation method.

A few methods with different sensitivities have been developed for determination of alkaloids in plant materials; for example, gravimetric and titrimetric methods. These methods lack the adequate sensitivity and have some problems. As with most gravimetric methods, the residue obtained is found to contain impurities since more than one spot is revealed by TLC. The titrimetric assay suffers from the disadvantage that the end-point is masked by the color of the extract. On the other hand, there is no constant method applicable for all alkaloids. Methods with high sensitivity such as HPLC are not routine methods for determination of total alkaloids and these methods are very costly and need special equipment. Spectrophotometric determination of total alkaloids with bromocresol green is a simple and sensitive method and does not need very special equipment. The proposed method has the advantage of being less time consuming, with the assay requiring an average of 1 h. The BCG can react with a certain class of alkaloids (alkaloids that have nitrogen inside their structure) and amine or amid alkaloids does not react with this reagent [15]. Therefore the method described in this study can be used for determination of a special group of alkaloids.

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Table 1 Determination of total alkaloids in tested plant materials (100 g) by BCG-complex formation.

No.	Plant	Part used	Amount (mg)	Amount (M mol)
1	*Acroptilon repens* L.	aerial parts	13.35	0.023
2	*Berberis vulgaris* L.	aerial parts	40.58	0.070
3	*Berberis vulgaris* L.	fruit	19.70	0.034
4	*Biebersteinia multifidia* DC.	aerial parts	204.56	0.353
5	*Biebersteinia multifidia* DC.	root	1688.47	2.920
6	*Calendula officinalis* L.	flower	16.14	0.028
7	*Chelidonium majus* L.	aerial parts	248.09	0.430
8	*Echium amoenum* Fish & Mey	flower	18.44	0.320
9	*Equisetum arvense* L.	aerial parts	255.02	0.440
10	*Hyoscyamus niger* L	aerial parts	324.09	0.560
11	*Hypecoum pendulum* L.	aerial parts	39.20	0.068
12	*Malva sylvestris* L.	aerial parts	35.06	0.060
13	*Scrophularia striata* Bioss.	root	7.90	0.014
14	*Stachys lavandulifolia* Vahl.	aerial parts	9.73	0.017

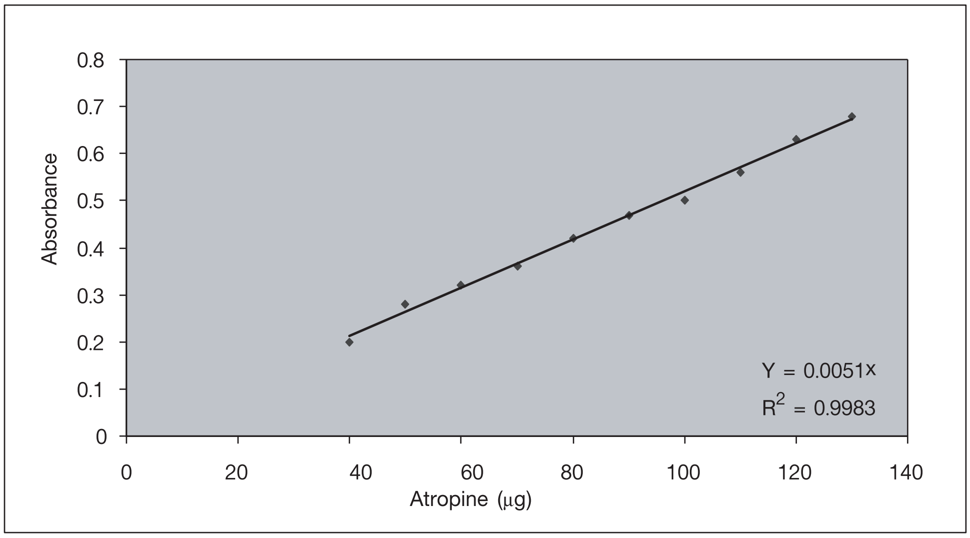

Figure 1 Variation of the absorbance with atropine concentrations at 470 nm

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