DETERMINATION OF BENZOIC ACID AND SALICYLIC ACID IN COMMERCIAL BENZOIC AND SALICYLIC ACIDS OINTMENTS BY SPECTROPHOTOMETRIC METHOD

IQBAL AHMAD* AND FAIYAZ HM VAID**

**Dubai Pharmacy College, P.O. Box 19099, Dubai, United Arab Emirates*
***Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Karachi-75270, Pakistan*

ABSTRACT

Commercial benzoic acid and salicylic acid ointments have been analyzed for benzoic acid and salicylic acid content by using a spectrophotometric method. Since benzoic acid and salicylic acid exhibit overlapping spectra, absorbance measurements are made at two wavelengths, i.e. 271 nm and 303 nm in ethanol (96%v/v) for simultaneous determination of the two compounds. The method is direct and involves only one step of absorbance measurement in the assay. The precision of the method, based on the analysis of synthetic mixtures of the two compounds, is within 2%. The recoveries of benzoic acid and salicylic acid in the ointments range from 99.9 to 100.6% and 99.5 to 101.3%, respectively and are comparable with those of the USP method (99.8 to 100.5% and 100.2 to 101.7%, respectively). The proposed method is simple, rapid, precise and convenient for the assay of benzoic acid and salicylic acid in commercial preparations.

Keywords: Benzoic acid; salicylic acid; spectrophotometric assay; commercial ointments.

INTRODUCTION

The official methods for the assay of benzoic acid and salicylic acid in compound benzoic acid ointment or benzoic and salicylic acids ointment are based on acid-base titration (benzoic acid) and spectrophotometric determination (salicylic acid) (BP, 2003) or column chromatographic separation of the two compounds followed by spectrophotometric determination (USP, 2004). Spectrofluorimetric (Adams and Miller, 1978) and isoabsorptive methods (Sethi, 1985) have been proposed for the assay of benzoic acid and salicylic acid in pharmaceutical preparations. These methods are tedious, time consuming and may be influenced by possible interference from related substances. Two/three-component spectrophotometric methods have been successfully applied to the simultaneous determination of pharmaceutical (Ahmad *et al.*, 1979; Ahmad *et al.*, 1980; Salinas *et al.*, 1990; Santoni *et al.*, 1990; Ahmad and Rapson, 1990; Heelis *et al.*, 1980; Ahmad *et al.*, 1992; Ahmad and Hussain, 1992; Garrido Frenich *et al.*, 1995; Gangwal and Sharma, 1996; Bhatia *et al.*, 1997; Mahgoub and Aly, 1998; Hund *et al.*, 1999; Ribone *et al.*, 1999; Dinc *et al.*, 2001; Dinc *et al.*, 2004; Wahbi *et al.*, 2003; Ahmad *et al.*, 2003; Ahmad *et al.*, 2004a; 2004b) and biological compounds (Zwart *et al.*, 1984; Shih *et al.*, 1997).

The object of this investigation is to develop a direct, simple, rapid, accurate, and precise spectrophotometric method for the simultaneous determination of benzoic acid and salicylic acid in commercial ointment preparations.

EXPERIMENTAL

Materials

Benzoic acid and salicylic acid (USP grade) were obtained from Sigma Chemical Co., St. Louis, MO. Ethanol (96% v/v) was analytical grade from BDH, Poole, Dorset, UK. All chemicals and reagents were analytical grade or of the purest form available from Sigma Chemical Co. / BDH.

Formulation

Whitfield's ointment containing 6% w/w benzoic acid and 3% w/w salicylic acid. Three different batches of the ointment were used in this study.

Equipment

A Beckman Du-70 microprocessor controlled spectrophotometer (Beckman Coulter, Fullerton, CA), was used for absorbance measurement using 1-cm quartz cells.

Method of Analysis

Extraction of benzoic acid and salicylic acid

A portion of the ointment sample equivalent to about 60 mg of benzoic acid and 30 mg of salicylic acid was warmed with 25 ml of ethanol (96% v/v) to melt the base and extracted with the solvent. It was further extracted

**Present address*: Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi.
**Corresponding author: Tel: 9261300-7, Ext 2203 / 3203, e-mail: fvaid7@yahoo.com

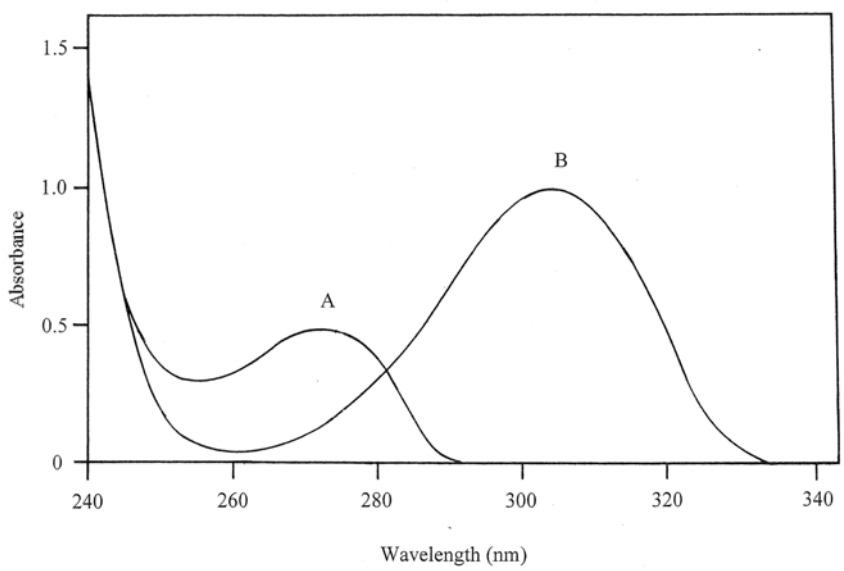

Fig. 1: UV absorption spectra of (A) benzoic acid (6.8 mg/dL) and (B) salicylic acid (3.8 mg/dL) in ethanol (96% v/v).

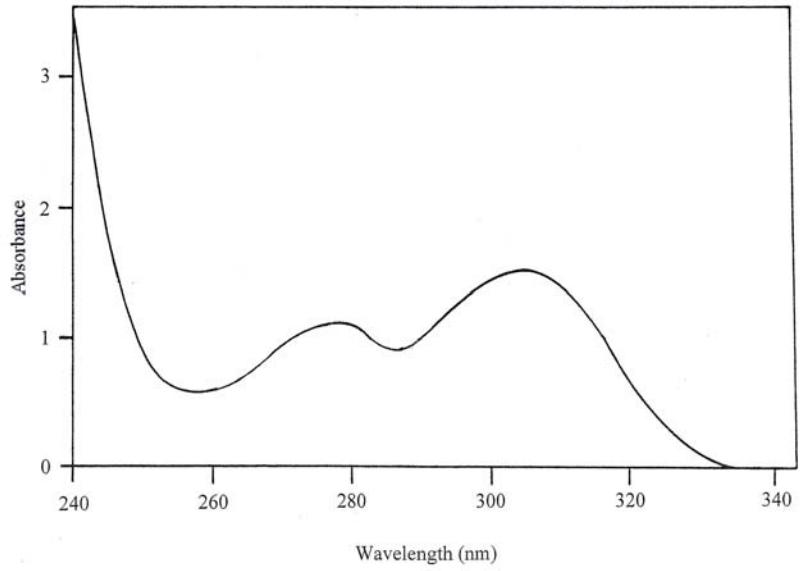

Fig. 2: UV absorption spectrum of ethanolic (96% v/v) extract of an ointment sample containing 0.012% w/w of benzoic acid and 0.006% w/w salicylic acid.

with two 25 ml aliquots of ethanol (96% v/v). The combined extracts were filtered and made up to 100 ml with ethanol (96% v/v).

Assay of benzoic acid and salicylic acid

A 5 ml aliquot of the extract was diluted to 50 ml in a volumetric flask with ethanol (96% v/v) and the absorbance measurements were carried out at two wavelength, i.e., 271 and 303 nm. The concentrations of benzoic acid and salicylic acid were determined by a two-component spectrophotometric assay using built-in software.

The assay involves the solution of two simultaneous equations (Fell, 1986):

$$A\lambda_1 = k_1^{\lambda.1} C_1 + k_2^{\lambda.1} C_2 \quad (1)$$

$$A\lambda_2 = k_1^{\lambda.2} C_1 + k_2^{\lambda.2} C_2 \quad (2)$$

Where $k_1^{\lambda.1}$ and $k_2^{\lambda.1}$ are the specific absorbance [A(1%, 1 cm)] values of component 1 and 2 at λ_1; $k_1^{\lambda.2}$ and $k_2^{\lambda.2}$ are the specific absorbance values of component 1 and 2 at λ_2; and C_1 and C_2 are the concentrations of component 1 and 2, respectively. The equations are solved to obtain the concentration of each component.

$$C_1 = \frac{k_2^{\lambda 2} A_{\lambda 1} - k_2^{\lambda 1} A_{\lambda 2}}{k_1^{\lambda 1} k_2^{\lambda 2} - k_2^{\lambda 1} k_1^{\lambda 2}} \quad (3)$$

$$C_2 = \frac{k_1^{\lambda 1} A_{\lambda 2} - k_1^{\lambda 2} A_{\lambda 1}}{k_1^{\lambda 1} k_2^{\lambda 2} - k_2^{\lambda 1} k_1^{\lambda 2}} \quad (4)$$

RESULTS AND DISCUSSION

Selection of analytical wavelengths

Important considerations in the selection of appropriate wavelengths for spectrophotometric determination of binary mixtures are maximum sensitivity (such as that at the absorption maxima), good adherence to Beer's law and minimum interference from instrumental factors (Christian, 1994). Factors influencing the selection of analytical wavelengths in multicomponent analysis have been studied and methods of their optimization have been proposed (Stearns, 1969; Knowles and Burgess, 1984; Frans and Harris, 1985; Sasaki *et al.*, 1986; Faber *et al.*, 2003).

The absorption spectra of benzoic acid and salicylic acid in ethanol (96% v/v) are sufficiently distinct to allow for the selection of their respective absorption maxima, i.e., 271 and 303 nm, for the analysis of the two compounds (fig. 1). At these wavelengths maximum specificity and sensitivity may be achieved for the analysis of these compounds.

Validity of Beer's law

The validity of Beer's law for benzoic acid and salicylic acid, in the concentration range 0.2 – 1.2 × 10^{-2} g/dL, alone or in mixtures, at the analytical wavelengths was established prior to the analysis. The values of specific absorbance [A(1%, 1 cm)] (table 1) used for the calculation of concentrations of benzoic acid and salicylic acid represent the means of determinations at atleast five different concentrations (correlation coefficients 0.998 – 0.999). These values are in agreement with those previously reported (Fell 1986; O'Neil, 2001).

Table 1: Specific absorbance [A(1%, 1 cm)] of benzoic acid and salicylic acid in ethanol (96%, v/v)*

Compound	271 nm	303 nm
Benzoic acid	66.4	0.35
Salicylic acid	33.0	260.2

*Each value is a mean of five to seven determinations.

Reproducibility of the assay method

The reproducibility of the assay method was tested by preparing several synthetic mixtures containing different proportions of benzoic acid and salicylic acid and analyzing for the two compounds by the proposed method. The results of the assay of benzoic acid and salicylic acid in mixtures are presented in table 2. Judging from the calculated values of percentage recovery, the reproducibility of the method appears to be within ± 2%. Therefore, the method may be considered reliable and precise for the assay of benzoic acid and salicylic acid in

Table 2: Analysis of synthetic mixtures of benzoic acid and salicylic acid*

Benzoic acid				Salicylic acid			
Added ($g \times 10^3$)	Found ($g \times 10^3$)	Recovery (%)	RSD (%)	Added ($g \times 10^3$)	Found ($g \times 10^3$)	Recovery (%)	RSD (%)
12.00	11.92	99.3	1.1	6.00	6.07	101.1	1.8
9.60	9.58	99.8	0.5	4.80	4.82	100.4	0.8
7.20	7.31	101.5	0.8	3.60	3.59	99.7	0.7
6.00	6.09	101.5	0.9	3.00	3.00	100.0	0.5
6.00	6.07	101.2	1.0	1.50	1.48	98.7	0.7
4.80	4.78	99.6	0.3	2.40	2.39	99.6	1.6
3.60	3.65	101.4	1.6	1.80	1.82	101.1	1.0
3.00	2.97	99.0	0.3	3.00	3.03	101.0	0.8

*Values expressed as a mean of three to five determinations.

Table 3: Assay results for benzoic acid and salicylic acid in the ointment*

Batch no.	Benzoic acid (6% w/w)			Salicylic acid (3% w/w)		
	Percent of label claim (g)			Percent of label claim (g)		
	Proposed method	USP method	E_r %	Proposed method	USP method	E_r %
1	99.9 ± 0.6	100.5 ± 1.2	- 0.6	101.3 ± 1.0	101.2 ± 1.7	+ 0.1
2.	100.4 ± 1.0	101.6 ± 1.7	- 1.2	100.9 ± 1.5	101.7 ± 1.5	- 0.8
3.	100.6 ± 1.2	99.8 ± 1.5	+ 0.8	99.5 ± 1.2	100.5 ± 1.9	- 1.0

*Mean ± SD, n = 3-5, E_r = relative error in spectrophotometric versus USP method.

commercial preparations.

Application of the method to the assay of ointments

The main object of the development of an analytical method is to establish optimum assay conditions to achieve a high degree of specificity, sensitivity and precision and its validation (Green, 1996). This has been achieved in the present study. The proposed method has been validated and applied to the assay of benzoic acid and salicylic acid in ointment preparations. The UV absorption spectrum of the ethanolic extract of an ointment sample is shown in fig. 2. The spectrum exhibits two absorption maxima at about 271 nm and 303 nm corresponding to a mixture of benzoic acid and salicylic acid which can be resolved by a two-component assay. The assay results obtained by the proposed method for benzoic acid and salicylic acid in three different batches of the ointment along with those obtained by the USP method are presented in table 3. The recoveries of benzoic acid and salicylic acid in the samples assayed by the proposed method are in good agreement with those of the USP method and the relative error of the proposed method versus USP method is within ± 1%. The method has the advantage of being simple, rapid and convenient involving only one step of absorbance measurements in the assay of benzoic acid and salicylic acid in commercial ointments.

CONCLUSION

A two-component spectrophotometric method has been developed for the direct simultaneous determination of benzoic acid and salicylic acid in commercial ointment preparations. The method is simple, rapid, accurate and precise for the assay of benzoic acid and salicylic acid. It is convenient for the determination of the two compounds in pharmaceutical preparations in the quality control laboratories.

REFERENCES

Adams S and Miller NSB (1978). The determination of salicylic acid and benzoic acid in pharmaceutical formulations by spectrofluorimetry. *J. Pharm. Pharmacol.*, **30**: 81-83.

Ahmad I, Ansari AA and Ismail T (2003). Effect of niotinamide on the photolysis of cyanocobalamin in aqueous solution. *J. Pharm. Biomed. Anal.,* **31**: 369-374.

Ahmad I, Fasihullah Q, Noor A, Ansari, IA and Ali QNM. (2004a). Photolysis of riboflavin in aqueous solution: a kinetic study. *Int. J. Pharm.*, **208**: 199-208.

Ahmad I, Fasihullah Q and Vaid FHM (2004b). A study of simultaneous photolysis and photoaddition reaction of riboflavin in aqueous solution. *J. Photochem. Photobiol., B: Biol*. **75**: 13-20.

Ahmad I and Hussain W (1992). Multicomponent spectrophotometric assay of cyanocobalamin, hydroxocobalamin and riboflavin. *Pak. J. Pharm. Sci.*, **5**(2): 121-127.

Ahmad I, Hussain W and Fareedi AA (1992). Photolysis of cyanocobalamin in aqueous solution. *J. Pharm. Biomed. Anal.*, **10**: 9-15.

Ahmad I, Khan MA, Usmanghani K and Salam T (1979). Spectrophotometric determination of hydrolytic products of reserpine. *Pharmazie.*, **34**: 402.403.

Ahmad I and Rapson, HDC (1990). Multicomponent spectrophotometric assay of riboflavin and photoproducts. *J. Pharm. Biomed. Anal.*, **8**: 219-223.

Ahmad I, Rapson HDC, Heelis PF and Phillips GO (1980). Alkaline hydrolysis of 7,8-dimethyl-10-(formylmethyl) isoalloxazine. A kinetic study. *J. Org. Chem.*, **45**: 731-733.

Bhatia MS, Karkhedikar SG and Chaturvedi SC (1997). Comparatives evaluation of different spectrophotometric methods developed for simultaneous estimation of diclofenic sodium, chlorzoxazone and paracetamol from combined dosage forms. *Indian Drugs*, **34**: 149-153.

British Pharmacopoeia (2003).The Stationary Office Limited, Norwich, UK, pp.2115-2116.

Christian GD (1994). Analytical Chemistry, fifth ed., Wiley, New York, pp.418-421.

Dinc E, Baleanu D and Onur F. (2001). Spectrophotometric multicomponent analysis of a mixture of metamazol, acetaminophen and caffeine in pharmaceutical formulations by two chemometric techniques. *J. Pharm. Biomed. Anal.*, **26**: 949-957.

Dinc E, Baleanu D, Ustundag O and Abdul-Enein HY (2004). Continuous wavelet transformation applied to the simultaneous quantitative analysis of two-component mixtures. *Pharmazie*, **59**: 618-623.

Faber NM, Ferre J, Boque R and Kalivas SH (2003). Quantifying selectivity in spectrophotometric multicomponent analysis. *Trends Anal. Chem*. **22**: 352-361.

Fell AF (1986). In: Moffat AC (Ed.) Clarke's Isolation and Identification of Drugs, second ed., The Pharmaceutical Press, London, pp. 227-228, 384, 965-966.

Frans SD and Harris JM (1985). Selection of analytical wavelengths for multicomponent spectrophotometric determinations. *Anal. Chem.*, **57**: 2680-2684.

Gangwal S and Sharma AK (1996). Simultaneous spectrophotometric determination of mefenamic acid and paracetamol in combined pharmaceutical dosage forms. *Ind. J. Pharm. Sci.*, **58**: 216-218.

Garrido Frenich A, Jouan-Rimbarrd D, Massart DL, Kuttatharmmakul S, Martinez Galera M and Martinez Vidal JL (1995). Wavelength selection method for multicomponent spectrophotometric determination using partial least squares. *Analyst.*, **120**: 2787-2792.

Green JM (1996). A practical guide to analytical method validation. *Anal. Chem.*, **68**: 305 A-30A.

Heelis PF, Phillips GO, Ahmad I and Rapson HDC (1980). The photodegradation of formylmethylflavin-A continuous and flash photolysis study, *Photobiochem. Photobiophys*., **1**: 125-130.

Hund E, Massart DL and Smeyers-Verbeke J (1999). Evaluation of the H-point standard additions method (HPSAM) and the generalized H-point standard additions method (GHPSAM) for the UV analysis of two-component mixtures. *J. Pharm. Biomed. Anal.*, **21**: 23-42.

Knowles A and Burgess C (Ed.) (1984). Practical Absorption Spectroscopy, Chapman and Hall, London, pp.167-168.

Mahgoub H and Aly FA (1998). UV spectrophotometric determination of ampicillin sodium and sulbactum sodium in two-component mixtures. *J. Pharm. Biomed. Anal*., **17**: 1273-1278.

O'Neil MJ (Ed.) (2001). The Merck Index, thirteenth ed., Merck & Co., Inc., New Jersey, p.1496.

Ribone ME, Pagani AP and Olivieri AE (1999). Simultaneous multivariate spectrophotometric analysis of binary and tertiary mixtures of sulfamethoxazole, trimethoprim and phenazopyridine in tablets. *Anal. Lett.*, **32**: 1389-1402.

Salinas F, Nevado JJB and Espinosa Mansilla A (1990). A new spectrophotometric method for quantitative multicomponent analysis, and resolution of mixtures of salicylic and salicyluric acids. *Talanta.*, **37**: 347-351.

Santoni G, Mura P, Pinzauti S, Lordardo E and Gratteri P (1990). Simultaneous UV spectrophotometric determination of procaine hydrochloride and phenazone in an otic formulation. *Int. J. Pharm.*, **64**: 235-238.

Sasaki K, Kawata S and Minemi S (1986). Optimal wavelength selection for quantitative analysis. *Appl. Spectrosc*., **40**: 185-190.

Sethi PD (1985). Quantitative Analysis of Drugs in Pharmaceutical Formulations. Unique Publishers, Delhi, pp.335-339.

Shih ML, Korte WD and Clark CR (1997). Multicomponent spectroscopic assay for hemoglobin and ferrihemoglobin species in methemoglobin treatments of cyanide poisoning. *J. Anal. Toxicol.*, **21**: 543-547.

Stearns EL (1969). The Practice of Absorption Spectrophotometry, Wiley-Interscience, New York, pp.131-135.

United States Pharmacopeia (2004). United States Pharmacopeia Convention, Rockville, MD, p.222.

Wahbi AM, Gazy AA, Abdel-Razak O, Mahgoub H and Moneeb MS (2003). Simultaneous determination of paracetamol and chlorzoxazone using orthogonal functions-ratio spectrophotometry. *Saudi Pharm., J.* **11**: 192-200.

Zwart A, Buursma A, Van Kampen EJ and Zijliztra WD (1984). Multicomponent analysis of hemoglobin derivatives with reversed-optics spectrophotometer. *Clin. Chem.*, **30**: 373-379.