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The determination of salicylic acid and benzoic acid in pharmaceutical formulations by spectrofluorimetry

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Methods of extraction from pharmaceutical formulations and subsequent determination of benzoic acid and salicylic acid by spectrofluorimetry are described. The recovery of benzoic acid, in the presence of salicylic acid, was 99·3%, with a coefficient of variation of 1·04%, while the recovery of salicylic acid, in the presence of benzoic acid, was 97·7%, with a coefficient of variation of 0·68%.

The determination of benzoic acid when formulated with salicylic acid in pharmaceutical preparations can be troublesome. The assay of benzoic acid in compound benzoic acid ointment of the British Pharmaceutical Codex (1973) relies on an indirect method. The salicylic acid is determined by its bromine absorption, (Kolthoff, 1921) and subsequently a correction for the calculated salicylic acid content is made in the titration of the total acid. Such a procedure suffers from possible interference (in some formulations) from either acids or substances that react with bromine. The fluorescence of salicylic acid in organo-chlorine solvents is enhanced by the presence of aliphatic carboxylic acids while benzoic acid fluoresces at a lower wavelength but less strongly than salicylic acid (Schenk, Boyer & others, 1972).

MATERIALS AND METHODS

Reagents

The benzoic acid and salicylic acid employed were of B.P. quality. Glacial acetic acid, hydrochloric acid and methanol were 'Pronalys' grade while iso-octane and diethylamine were reagent grade (May and Baker Ltd, Dagenham). Dichloromethane was glass distilled, suitable for fluorimetry (Rathburn Chemicals, (Walkerburn) Ltd, Peebleshire).

The fluorimetric solvent consisted of 1% glacial acetic acid in dichloromethane.

Instrumentation settings

The instrument used was a Perkin Elmer MPF-3 spectrofluorimeter. The settings for the determination of benzoic acid were as follows: excitation wavelength, 282 nm; slit width 7, emission wavelength, 305 nm; slit width, 7; sensitivity 10. The settings for the determination of salicylic acid were as follows; excitation wavelength, 315 nm; slit width, 7; emission wavelength, 445 nm, slit width, 7, and sensitivity, 3.

Extraction of analytes

(a) *Creams and ointments.* A portion of the sample, equivalent to 100 mg benzoic acid and/or 100 mg salicylic acid, was accurately weighed into a 100 ml separator and dispersed in 40 ml iso-octane.

The solution was extracted with three 25 ml aliquots of methanol which were bulked and made up to 100 ml with methanol.

(b) *Aqueous solutions.* A suitable volume, equivalent to approximately 100 mg benzoic acid and/or salicylic acid, was pipetted into a 100 ml separator, made acid with dilute hydrochloric acid and extracted with three 25 ml aliquots of dichloromethane. The combined extracts were made up to 100 ml with dichloromethane.

Analysis

5 ml of the extract was pipetted into a 200 ml volumetric flask and diluted to volume with fluorimetric solvent (Solution A).

(a) Salicylic acid: 5 ml of solution A was pipetted into a 100 ml volumetric flask and diluted to volume with the fluorimetric solvent. The relative fluorescence of this solution was measured under the conditions described and compared with that of a standard solution of salicylic acid (1·25 μg ml^{-1}).

(b) Benzoic acid: Five 2 ml aliquots of Solution A were pipetted into each of five 50 ml volumetric flasks containing 0, 1, 2, 3 and 4 ml benzoic acid standard solution (25 μg ml^{-1}) and made up to volume with the fluorimetric solvent. The relative fluorescence intensities, of the solutions, 4 ml in a 10 mm silica cuvette, were measured, under the conditions described, before and after the addition of 3 drops of diethylamine. The second reading was

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subtracted from the first reading and a graph constructed of the difference fluorescence reading versus the concentration of added standard. The concentration of benzoic acid in the sample solution, and hence in the original product, was determined from the intercept on the concentration axis when the plotted points were extrapolated.

Fluorescence quenching experiments

(i) A solution of benzoic acid (25 μg ml^{-1}) in the fluorimetric solvent was prepared. 2 ml aliquots were pipetted into each of four 50 ml volumetric flasks to which was added 0, 10, 15 and 20 ml of a solution of salicylic acid in the fluorimetric solvent, (25 μg ml^{-1}) and made up to volume with the solvent. The fluorescences of the solutions were read under the conditions previously described for the determination of benzoic acid. From the data, plots (Fo/F)-1 *vs* [Q] and (Fo/F-1)[Q] *vs* [Q] were constructed. Fo is the fluorescence of the solution without salicylic acid, F is the fluorescence of the solution with salicylic acid added, and [Q] is the concentration of added salicylic acid.

(ii) The following solutions were prepared.

(a) benzoic acid solution in the fluorimetric solvent (50 μg ml^{-1});

(b) as (a) with (200 μg ml^{-1}) salicylic acid added;

(c) salicylic acid solution in the fluorimetric solvent (200 μg ml^{-1}).

The fluorescence of solutions (a) and (b) were measured at the excitation and emission maxima for benzoic acid. The fluorescences of solutions (b) and (c) were measured at the excitation and emission maxima for salicylic acid. The fluorescences of solutions (b) and (c) were measured at the emission maximum for salicylic acid and excited at the excitation maximum for benzoic acid.

RESULTS AND DISCUSSION

Benzoic acid exhibits very weak fluorescence in dichloromethane but with the addition of glacial acetic acid there is a 14-fold increase in its relative fluorescence intensity, making its analysis in the presence of salicylic acid feasible since their emission maxima are well separated (Fig. 1). However, salicylic acid quenched the fluorescence of benzoic acid and vice versa but there was no increase in the fluorescence of salicylic acid in the presence of benzoic acid, when excited at the wavelength of maximum excitation for benzoic acid. This would tend to indicate that trivial processes of quenching were not involved e.g. reabsorption of the benzoic acid fluorescence by salicylic acid molecules with the

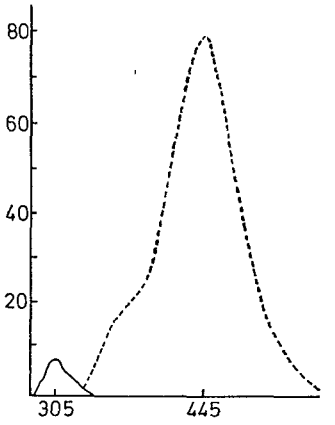

FIG. 1. The relative fluorescence intensity (ordinate) of benzoic acid (—) excited at 282 nm and the relative fluorescence intensity of salicylic acid (– – –) excited at 315 nm. Abscissa: Emission wavelength (nm).

subsequent emission of fluorescence characteristic of salicylic acid. Also, a radiationless transfer mechanism, by association of the molecules, was discounted since a plot of (Fo/F)-1 *vs* the added quencher concentration exhibited no deviation from a straight line (Fig. 2). This plot is based on the Stern-Volmer (1919) relation where

$$Fo/F = 1 + Kq[Q]$$

and Kq is the quenching constant. A plot (Fo/F)-1/[Q] gave a straight line with zero slope which according to Moon, Poland & Scheraga (1965) indicated that collisional processes are involved. According to these authors this is a more sensitive means of detecting association of the molecules, which is manifested by deviation from a zero slope.

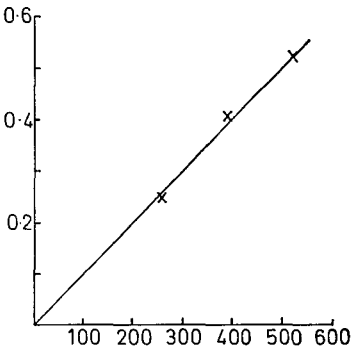

FIG. 2. The Stern-Volmer plot for benzoic acid at a concentration of 1 μg ml^{-1}, in the presence of increasing amounts of quencher (salicylic acid). Ordinate—(Fo/F) —1. Abscissa: Salicylic acid concentration (μg per 50 ml).

Since mutual quenching occurs it has to be corrected by the use of the standard addition technique for the analysis of benzoic acid. This technique overcomes errors of a proportional nature. The quenching of salicylic acid by benzoic acid was negligible at the concentrations used so that salicylic acid could be determined directly.

A sample of compound benzoic acid ointment, B.P.C., was analysed for its benzoic acid content by the method but without diethylamine. Thus, the unquenched benzoic acid fluorescence plotted against added standard concentration gave a straight line but high results were obtained, suggesting that some other component, co-extracted with the organic acids, caused an appreciable background fluorescence. By addition of diethylamine to the sample solution only the benzoic acid fluorescence was quenched. Thus, by measuring the difference between the original fluorescence and the fluorescence on the addition of diethylamine a result consistent with the stated label strength was obtained.

Using this technique, benzoic acid in a solution of benzoic and salicylic acids of known strength (1% of each) was assayed. The salicylic acid was also determined but, in this case, the interference from background fluorescence had a negligible effect and the addition of diethylamine was not necessary. Six replicates of benzoic acid and salicylic acid gave results of good accuracy and reproducibility. The recoveries were 99·3 and 97·7% and the coefficients of variation were 1·04 and 0·68% for benzoic acid and salicylic acid respectively.

Having ascertained the accuracy and precision of the method on a solution of known composition the method was used to determine the acids in a number of pharmaceutical formulations and, where applicable, compared with the results obtained by means of compendial methods (Tables 1 and 2). The recoveries of salicylic acid and benzoic acid in two 'official' ointments were in good agreement with the compendial titration method and the recoveries in the other preparations were good with one exception. In the case of the proprietary cream a low benzoic acid content was found but this was likely to be a true result since the absorbance of an extract was less than that of a standard solution of salicylic acid and benzoic acid made up to the same concentration. The official analytical technique could not be applied to this cream since another organic acid was present that interfered with the titration method.

The separation of the acids from ointment and cream base by partition between methanol and iso-octane gave an emulsion-free, clean separation and allowed for the rapid accurate and precise determination of benzoic acid and salicylic acid in the same preparation by spectrofluorimetry.

Table 1. *Comparison of the proposed method with the official method for the determination of salicylic acid in the presence of benzoic acid in a number of pharmaceutical formulations.*

Product	Salicylic acid content (%)	Proposed procedure Found	Proposed procedure Recov. (%)	B.P.C. method Found	B.P.C. method Recov. (%)
Co. benzoic acid ointment A	3·0	3·15	105·0	3·04	101·3
Co. benzoic acid ointment B	3·0	3·20	106·7	3·23	107·7
Proprietary cream	0·6	0·63	105·0	—	—
Co. glycerin of thymol A	0·49	0·47	95·9	—	—
Co. glycerin of thymol B	0·49	0·48	98·0	—	—

Table 2. *Comparison of the proposed method and the official method for the determination of benzoic acid in the presence of salicylic acid in some pharmaceutical products.*

Product	Benzoic acid content (%)	Proposed method Found	Proposed method Recov. (%)	B.P.C. method Found	B.P.C. method Recov. (%)
Co. benzoic acid ointment A	6·0	5·78	96·3	5·83	97·2
Co. benzoic acid ointment B	6·0	5·71	95·2	5·80	96·7
Proprietary cream	1·0	0·83	83·0	*	—
Co. glycerin of thymol A	0·68	0·75	110·3	—	—
Co. glycerin of thymol B	0·64	0·68	106·3	—	—

* See text.

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