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Comparison of reversed-phase liquid chromatography with colorimetry for analysis of phenolphthalein preparations

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Abstract

The high-performance liquid chromatography (HPLC) method of the United States Pharmacopeia has been compared with the colorimetric method of the British Pharmacopoeia for the assay of phenolphthalein in various preparations. Results are presented for the linearity, sensitivity and reproducibility of the two methods. The HPLC method was considered to be more convenient for routine analysis of the preparations of phenolphthalein.

Keywords: Phenolphthalein; Reversed-phase chromatography; Colorimetry

1. Introduction

Phenolphthalein, 3,3-bis(*p*-hydroxyphenyl-phthalide), is a drug of the group of stimulant laxatives [1]. It is used in a variety of formulations in which it is incorporated as the sole active principle or combined with other laxative agents (anthraquinones such as aloes) [2].

The quantification of phenolphthalein as the raw material or in tablets can be achieved by different methods. The USP XXI [3] described an iodometric method for its determination as the raw material and a gravimetric method for the drug in tablets. The USP XXII and the USP XXIII [4,5] specify a high-performance liquid chromatography (HPLC) method for the determination of phenolphthalein as the raw material and in tablets. the BP [6] specifies a spectrophotometric method for the drug substance only; there are no formulated preparations of phenolphthalein in the BP [6].

In this study, the two official methods are compared: HPLC according to the USP XXIII [5] and spectrophotometry according to the BP [6].

2. Experimental

2.1. *Materials*

Phenolphthalein, methanol and acetic acid PRS were purchased from Merck (Darmstadt, Germany). Aloin and glycine were from Sigma (St. Louis, MO, USA). Distilled de-ionized water was used in the preparation of all aqueous solutions.

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2.2. Formulations

A. Laxative granulate "El Aleman" (Batch G-1). Phenolphthalein 580 mg g^{-1}.

B. "Pildoras Zeninas" (Batch G-15). Phenolphthalein 40 mg and aloes 60 mg per pill.

C. Dragees "Laxante Salud" (Batch G-2). Phenolphthalein 50 mg and aloes 50 mg per dragee.

D. Mineral oil emulsion "Emuliquen Laxante" (Batch H-2). Phenolphthalein 47 mg and liquid paraffin 2.5 ml per 5 ml.

2.3. Equipment

The HPLC system comprised two Gilson (Middleton, WI, USA) 305 and 306 pumps and a Gilson 231XL automatic sampler attached to a Rheodyne injection valve (20-μl sample loop). Detection of the analytes was accomplished using a gilson 116 variable-wavelength UV detector. Data were recorded on a Spectra-Physis SP4270 integrator (San Jose, CA, USA). A Beckman DU-6 spectrophotometer was used in the colorimetric method.

2.4. Chromatographic conditions

The mobile phase was methanol–water–glacial acetic acid (50:50:1, v/v/v). The mobile phase was filtered through a 0.45-μm Millipore filter prior to use. A 100 × 4 mm i.d. column packed with 7-μm Nucleosil C_{18} was used; the flow-rate was 1.5 ml min^{-1}. The injection volume was 20 μl and ultraviolet detection was at 280 nm (0.2 aufs).

2.5. Sample preparation

HPLC method

The equivalent of 500 mg of phenolphthalein from the sample was transferred to a 100-ml volumetric flask and diluted to 100 ml with methanol. The mixture was sonicated for 5 min and filtered through a 0.45-μm millipore filter; 5 ml of the filtrate was transferred to a 100-ml volumetric flask and diluted to 100 ml with methanol. The injection volume was 20 μl.

Spectrophotometric method

The equivalent of 100 mg of phenolphthalein from the sample was transferred to a 100-ml volumetric flask and diluted to 100 ml with ethanol (96%, v/v). The mixture was sonicated for 5 min and filtered through a 0.45-μm Millipore filter; 5 ml of the filtrate was transferred to a 50-ml volumetric flask and diluted to 50 ml with ethanol (96%, v/v). Then 5 ml of the resulting solution was then evaporated to dryness; the residue was dissolved in glycine buffer (pH 11.3) and diluted to 100 ml with the same buffer. The absorbance at 555 nm of the resulting solution was immediately measured.

2.6. Calibration curves for phenolphthalein

Working solutions containing 0–75 μg ml^{-1} (HPLC method) and 0–8 μg ml^{-1} (spectrophotometric method) were prepared just before assaying.

2.7. Treatment of analytical data

The resolution between two chromatographic peaks (R) was calculated from

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2} \quad (1)$$

where t_1 and t_2 are the retention times, and W_1 and W_2 the respective widths of the peaks.

The confidence limits for the slope and intercept of the line of regression were calculated by the equations:

$$b \pm tS_b \quad (2)$$

$$a \pm tS_a \quad (3)$$

where t is the value of Student's-t at $P = 0.05$ for $n - 2$ degrees of freedom, and S_a and S_b are the standard error of the intercept (a) and the standard error of the slope (b), respectively. If zero lies between the confidence limits for a the proportionality condition is achieved.

Linearity was evaluated by calculation of the relative standard deviation of the slope ($S_{b\,rel}$ %) according to the following equation:

$$(S_{b\,rel}\ \%) = \frac{S_b}{b}\, 100 \quad (4)$$

where S_b is the standard deviation of the slope (b).

Detection limits (DLs) were calculated from the equation:

$$\mathrm{DL} = \left(S_0^2 \frac{n-2}{n-1}\right)^{1/2} \cdot \frac{t_p}{b} \quad (5)$$

where n is the number of samples, t_p is the value of Student's-t at $P = 0.05$ and $(n - 2)$ degrees of freedom, b is the gradient and S_0^2 is the variance characterizing the dispersion of the points with respect to the line of regression.

The limit for experimental detection is the lower concentration that can be found with a relative standard deviation (RSD) lower than the limit specified by the pharmacopoeia. The RSD value for phenolphthalein according to the USP XXIII [5] is <2%.

The analytical recovery was calculated from 100 × amount found/amount added.

3. Results and discussion

The composition of the mobile phase of methanol–water–glacial acetic acid (50:50:1, v/v/v) allowed good separation between the peaks for phenolphthalein (PT) and aloin (A) (the active principle used for aloe quantification). Fig. 1 shows HPLC chromatograms of phenolphthalein and aloin with retention times of 3.86 min and 8.04 min, respectively. The resolution between chromatographic peaks for phenolphthalein and aloin was $R = 3.86$. This value allows good resolution without interference from aloin in the analysis of commercial formulations (B and C) phenolphthalein and aloes.

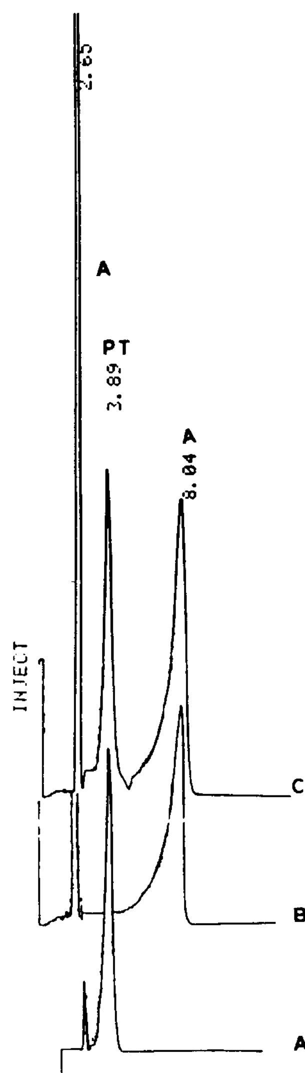

Fig. 1. HPLC chromatograms of phenolphthalein (PT) and aloin (A) from formulations A, B and C, with their retention times (min).

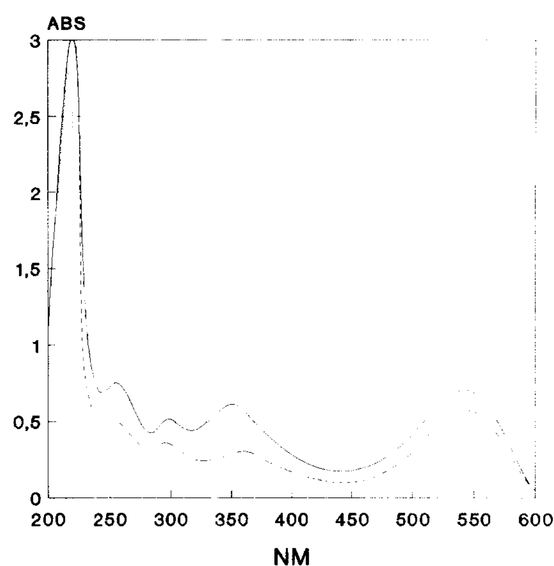

Fig. 2. Spectrophotometric scans corresponding to phenolphthalein (in methanol) as the raw material (· · ·) and as an active principle in pharmaceutical formulations B (——) and C (– – –).

Fig. 2 shows spectrophotometric scans corresponding to phenolphthalein as the raw material and as an active principle in pharmaceutical formulations (B and C). There was no interference from aloin for the measurement at 555 nm.

The gradients and intercepts of the calibration curves and the linearity of each calibration graph are shown in Table 1. The intercept values were not statistically different from zero ($P < 0.05$). The HPLC method showed a statistical difference from zero ($P < 0.05$) in the intercept values.

The values for the correlation coefficient were not sufficient to evaluate the linearity of the calibration grpahs. The linearity was determined by the RSD of the slope ($S_{b\,rel}$ %) [7]. The linearity was better in the HPLC method ($S_{b\,rel}$ % = 1.75) than in the spectrophotometric method ($S_{b\,rel}$ % = 3.37).

The concentration range, detection limit and relative sensitivity of the different methods are shown in Table 2. The concentration range for

Table 1
Inter-day reproducibility ($n = 3$) according to different methods of determination

Analysis method	Slopes [a] (cm^{-1} $\mu g\ ml^{-1}$)	Intercept [b]	Linearity $S_{b\ rel}$ [c] (%)
HPLC	1984.27 ± 149.87	30190.91 ± 8431.4	1.76
Spectroscopy	0.0925 ± 0.0087	0.0212 ± 0.0456	3.38

[a] Confidence limits of the slope ($P = 0.05$).
[b] Confidence limits of the intercept ($P = 0.05$).
[c] $S_{b\ rel}$ (%) is the RSD of the slope.

Table 2
Concentration range, detection limit and relative sensitivity of the two methods

Analysis method	Concentration range ($\mu g\ ml^{-1}$)	Detection limit ($\mu g\ ml^{-1}$) (calculated/experimental)	Relative sensitivity [a]
HPLC	35–100	3.60/4.00	40
Spectroscopy	0–10	0.51/0.01	1

[a] Calculated in relation to the experimental detection limit of the spectroscopic method.

Table 3
Inter-day reproducibility ($n = 3$) according to different methods of determination

Analytical method	concentration ($\mu g\ ml^{-1}$)	RSD (%)
HPLC	35	1.08
	50	1.35
	75	0.59
Spectroscopy	1.5	3.50
	5	4.78
	7	3.74

the HPLC method was 35–100 $\mu g\ ml^{-1}$. The concentration range for the spectrophotometric method was 0–10 $\mu g\ ml^{-1}$. The detection limits for the HPLC method evaluated by the statistical method were similar to those calculated according to the experimental method. In the spectrophotometric method the detection limits calculated by the statistical method (0.51 $\mu g\ ml^{-1}$) were higher than those calculated by the experimental method (0.1 $\mu g\ ml^{-1}$). These results were due to the poorer linearity of the spectrophotometric method.

Table 4
Analytical results for commercial formulations

Formulation	% Phenolphthalein theoretical	Analytical method (mean ± SD) spectroscopy HPLC	Difference
A	0.90	0.990 ± 0.32 0.927 ± 0.47	NS [a] ($P > 0.05$)
B	28.57	35.700 ± 0.86 29.18 ± 0.87	SS [b] ($P < 0.01$)
C	10.80	13.14 ± 0.46 10.35 ± 0.50	SS [b] ($P < 0.05$)
D	0.95	0.98 ± 0.06 0.92 ± 0.05	NS [a] ($P > 0.05$)

[a] NS = not significant.
[b] SS = statistically significant.

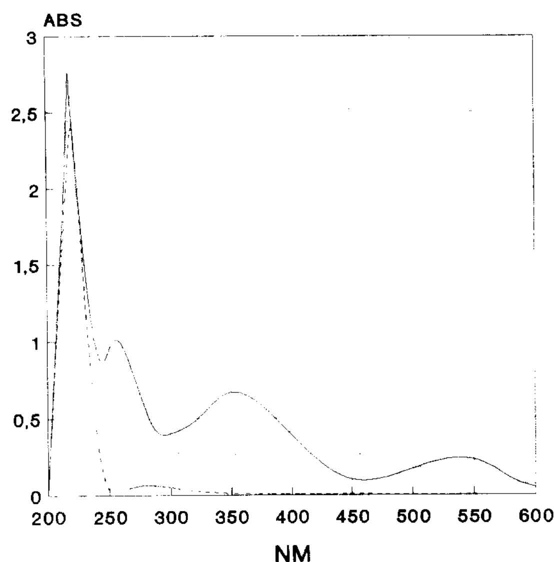

Fig. 3. Spectrophotometric scans corresponding to phenolphthalein (in acetic acid 1% v/v in methanol) as the raw material ( ) and as an active principle in pharmaceutical formulations B (···) and C ( ).

The inter-day reproducibility ($n = 3$) of the different methods for the determination of phenolphthalein is shown in Table 3. Both methods were reproducible. The RSD obtained was < 1.31% for the HPLC method; a slightly larger value was obtained by Wilhelm (RSD < 2.97%) [8].

All the different commercial formulations were evaluated by both methods. Assay results are shown in Table 4. No significant differences at the 95% confidence level (Student-*t* test and F-Snedecor) were found between the results obtained by HPLC and those by spectrophotometry for the determination of phenolphthalein determination in formulation A. However significant differences ($P < 0.05$) between the methods were found for formulations B and C; in these compound preparations phenolphthalein is formulated with other active principles. In these cases, the HPLC method showed values close to the theoretical values because HPLC is a separative method that avoids interference from other components. Fig. 3 shows the interference produced in the formulation B and C for the spectrophotometric method. To avoid interference, acetic acid (1% v/v) in methanol was used as a solvent: all samples were extracted with this solvent in order to remove the other active principles and excipients. This modified method enabled quantification of phenolphthalein to be achieved without interference (Table 5). These values were similar to those obtained by the HPLC method.

The analytical recoveries calculated by adding phenolphthalein to all formulations (A, B, C and D) were 103.6, 102.1, 98.8 and 104.8%, respectively, for the HPLC method; recoveries of 100.8, 104.9, 101.6 and 106.8%, respectively, were obtained for the spectrophotometric method. These results comply with the USP and BP requirements (tolerance 90–110%).

4. Conclusions

The HPLC method (USP) and the spectroscopic (BP) method were shown to be reproducible and sensitive in the analysis of phenolphthalein in the raw material.

Under experimental conditions, spectroscopy was the most sensitive method; however, the best linearity expressed as RSD and inter-day reproducibility were obtained with the HPLC method.

In commercial preparations in which phenolphtalein is associated with other active prin-

Table 5
Analytical results for HPLC and the modified colorimetric method [a]

Samples	% Phenolphthalein theoretical	Analytical method (mean ± SD) spectroscopy HPLC	Difference
B	28.57%	30.06 ± 0.62 29.14 ± 0.72	NS [b] ($P > 0.05$)
C	10.80%	10.86 ± 0.24 10.43 ± 0.70	NS [b] ($P > 0.05$)

[a] Modified method in which samples were extracted with acetic acid (1% v/v) in methanol to remove interfering substances.
[b] NS = not significant.

ciples and/or excipients, the preferred method is HPLC that avoids interference from the other components.

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