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Comparison of quantitative high performance thin layer chromatography and the high performance liquid chromatography of parabens

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Abstract

A method is described for the densitometric determination of the *p*-hydroxybenzoic esters and *p*-hydroxybenzoic acid in mixtures or in drugs. This method is compared with the one used in high performance liquid chromatography (HPLC). The calibration curves were linear in interval 0.250–3.60 μmol ml^{-1} per 200 nl per spot.

The limit of detection and the relative standard deviation (RSD) are higher than in HPLC (RSD is 6% in HPTLC, 3% in HPLC; limit of detection about 40 pmol in HPTLC and 25 pmol in HPLC) but HPTLC quantitative determination of parabens in drugs is faster.

Keywords: *p*-Hydroxybenzoic esters; High performance thin layer chromatography; High performance liquid chromatography; Rapid quantitative analysis method

1. Introduction

Some *p*-hydroxybenzoic esters (parabens) have been used as preservatives in food and drugs for about 70 years. The pharmaceutical formulations always contain low concentrations of these esters, so they are often analysed by chromatography. A large number of these analyses have been published for HPLC [1–7,15] and gas chromatography (GC) [8–11]. The thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) have also been applied to the determination of the esters [12–14,16].

In recent years, HPTLC has found growing acceptance and application among the modern separation techniques. The aim of this study was to develop a rapid quantitative reversed phase (RP) HPTLC method to quantify the four parabens and their degradation product *p*-hydroxybenzoic acid. The results were compared with those of RP-HPLC to see if the proposed method can be used for the determination of the *p*-hydroxybenzoic esters and *p*-hydroxybenzoic acid in two different pharmaceuticals, a healing foam and an antiseptic suspension ear drops.

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2. Experimental

2.1. Chromatographic apparatus

In TLC a Camag ATS III auto sampler, a Camag TLC scanner II and a Camag rectangular chamber (10 × 10 cm^2) (Merck, Nogent-Sur-Marne, France) were used.

The HPLC system consisted of a Waters pump 6000A (Waters, Saint-Quentin en Yvelines, France), a Rheodyne injection valve 7161 fitted with a 20 μl sample loop (Interchim, Montluçon, France) and a 4500 diode array detector (Merck).

2.2. Reagents

The separations were carried out on 10 × 10 cm^2 precoated glass HPTLC RP_{18} F_{254} plates from (Merck) or an HPLC Lichrospher RP_{18} column (dp: 5 μm, length: 11.9 cm, i.d.: 0.40 cm) (Merck).

p-Hydroxybenzoic acid (PHBA) (Merck), methyl *p*-hydroxybenzoate (MPHB), ethyl *p*-hydroxybenzoate (EPHB), propyl *p*-hydroxybenzoate (PPHB) and butyl *p*-hydroxybenzoate (BPHB) were purchased from Sigma (Saint-Quentin, Fallavier, France). HPLC grade methanol and acetic acid were provided by Carlo ERBA (Rueil Malmaison, France) and Merck, respectively.

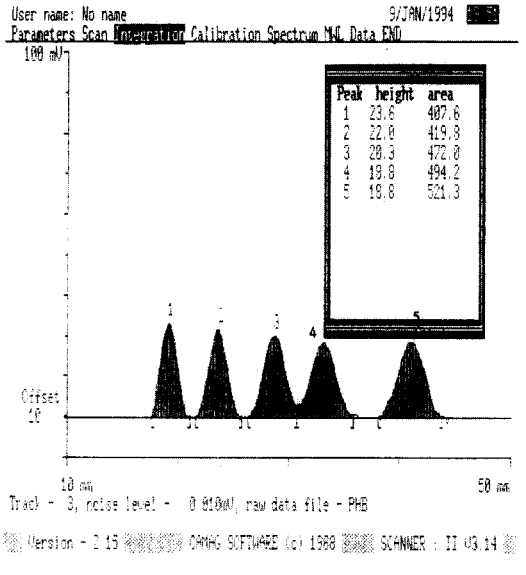

Fig. 1. The HPTLC chromatogram: BPHB; PPHB; EPHB; MPHB; and PHBA.

2.3. Samples

The nine standard solutions of five compounds with concentrations in the range 0.05–50.00 μmol ml^{-1} were prepared by dilution in methanol and used to plot the calibration graph in HPTLC and in HPLC.

The solutions of pharmaceuticals were prepared by dissolving 2 g foam in methanol in a 10 ml volumetric flask and 2 g ear drops in methanol in a 10 ml volumetric flask. Both solutions were injected or applied on plates without extraction and there were no interference with other compounds of the foam or of the ear drops suspension. Together with excipients, the foam contained methanol and calendula and nystatine and crotamidon were present in the ear drops suspension. The choice of methanol as sample solvent only depended on the paraben solubility and was independent on all other compounds in the drug; this solvent was able to extract the parabens from other compounds and was eliminated by spot drying in HPTLC.

2.4. Experimental conditions

The RP_{18} stationary phase was chosen to compare the paraben HPLC and HPTLC separation. This stationary phase has already been suggested, in Phamacopoeia to separate and to identify the related compounds of methylparahydroxybenzoate [12].

In HPTLC the thin layer was divided into two equal parts measuring 10 × 5 cm^2, a double number of samples were applied to the opposite wide sides of the plates. 200 nl of methanolic ester solution were applied with ATS, in spray, on the plates along a straight line 10 mm above the rim of the plate. The spots were spaced at a

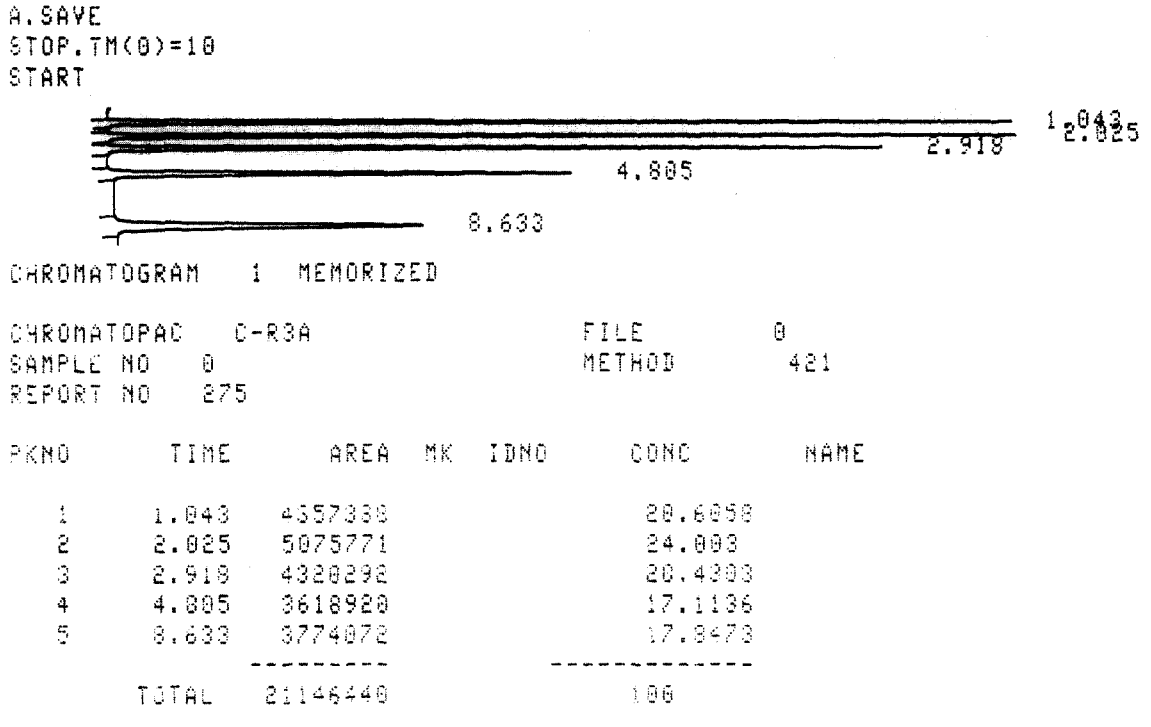

Fig. 2. The HPLC chromatogram: PHBA; MPHB; EPHB; PPHB; and BPHB.

be a more suitable vehicle for this ingredient than distance of 10 mm from one another then they were developed twice at room temperature in a rectangular Camag chamber. A mobile phase with the same solvents: methanol, water and acetic acid were used in the same target [16]. The first development was carried out with a methanol:water:acetic acid mixture (7:3:0.1 v/v/v) with a 2 cm developing path. After drying, they were again developed with a methanol:water:acetic acid mixture (6.5:3.5:0.1 v/v/v) with a 3.5 cm developing path. Then they were dried for 10 min at 50°C.

A quantitative determination by measuring the absorbance of the spots with the Camag TLC II scanner was carried out at 260 nm (lamp D_2), monochromator bandwidth 10 nm, slit dimensions 5/0.5 mm and scanning speed of 10.0 mm s^{-1}.

In HPLC the mobile phase was a methanol:water mixture (60:40, v/v) and the flow-rate 1 ml min^{-1}; 20 μl of each solution was injected into the chromatograph and the quantitative determination was made at 254 nm.

The detection limit of each compound was determined with the peak area equal to three times the background and the quantification limit was considered to be 10/3 of the detection limit.

3. Results and discussion

In RP_{18}HPTLC, 2 developments were carried out. The first one began the separation with low R_f values, the aim of the second one was to increase these R_f values. The mobile phase composition was modified to achieve the separation of the most used parabens.

The two developments were sufficient to achieve good separation of the four esters and the PHBA for identification and determination of

Table 1
Repeatability of HPTLC analysis and HPLC analysis

	M_w[a]	HPTLC[b] (one plate, 6 spots, $n = 10$[c])		HPTLC[d] (60 spots on 10 plates, $n = 1$)		HPLC ($n = 15$)	
		R_f[e]	RSD[f] (%)	R_f mean	RSD (%)	t_R[g] (min)	RSD (%)
BPHB	194.22	0.25	1.9	0.24	7.6	8.57	0.8
PPHB	180.20	0.37	2.1	0.36	8.6	4.90	0.8
EPHB	166.17	0.48	1.6	0.48	6.4	3.06	0.9
MPHB	152.14	0.60	1.5	0.61	4.6	2.17	0.9
PHBA	138.12	0.87	1.2	0.87	1.5	1.20	0.9

[a] Molecular weight.
[b] On 1 plate.
[c] Experiment number.
[d] On different plates.
[e] Retardation factor.
[f] Relative standard error.
[g] Retention time.

Table 2
HPTLC linear regression, limit of detection and repeatability of paraben analysis

Parabens	Linearity range (μmol ml^{-1})		Regression curve				r[e]	Limit of detection (pmol)	Repeatability ($n = 5$)
	Lower limit	Upper limit	a[a]	S_a[b]	b[c]	S_b[d]			RSD[f] (%)
BPHB	0.206	3.609	39.58	1.9	220.48	78.6	0.993	40	5.1
PPHB	0.222	3.889	41.69	1.8	190.46	74.7	0.994	45	5.6
EPHB	0.241	4.217	44.21	2.1	197.32	83.2	0.994	50	5.7
MPHB	0.260	4.605	46.86	2.2	161.39	89.1	0.993	50	5.6
PHBA	0.192	3.365	52.01	1.7	76.63	68.3	0.997	40	8.3

[a] Slope.
[b] Standard deviation of the slope.
[c] Intercept.
[d] Standard deviation of the intercept.
[e] Correlation coefficient of the fits.
[f] Legends as indicated in Table 1.

small quantities (Fig. 1). In HPLC all the peaks were separated. The retention times for PHBA, MPHB, EPHB, PPHB, BPHB were respectively 1.20, 2.17, 3.06, 4.90 and 8.57 min (Fig. 2).

The two methods were validated in term of repeatability for the R_f in HPTLC on one plate, and on different plates and for the retention time (t_R) in HPLC. The results are shown in Table 1. For the repeatability of the determination, the experiments were done on five samples and every sample was chromatographed three times and on one column.

The repeatability was slightly better in HPLC than in HPTLC (Tables 2 and 3). The mean RDS in HPLC was 3% whereas it was 6% in HPTLC.

The methods were also validated in terms of linearity, repeatability and limit of detection. The calibration curves (peak area versus concentrations in paraben solutions) were calculated

Table 3
HPLC linear regression, limit of detection and repeatability of paraben analysis

Parabens	Linearity range (μmol ml^{-1})		Regression curve					Limit of detection (pmol)	Repeatability ($n = 15$)
	Lower limit	Upper limit	a[a]	S_a[b]	b[c]	S_b[d]	r[e]		RSD[f] (%)
PHBA	12.9	104×10^3	379 188	4875	27 633	157 571	0.999	19	3.7
MPHB	9.4	76×10^3	220 072	10 609	265 792	342 941	0.997	30	2.7
EPHB	10.3	83×10^3	271 891	3231	76 664	104 436	0.999	25	4.5
PPHB	10.2	90×10^3	242 968	4988	117 036	161 231	0.999	26	1.4
BPHB	12.0	97×10^3	219 080	17 897	649 041	578 536	0.990	30	2.6

Footnotes are as in Table 2.

using the least square regression method. Their equations were determined from the five concentrations and the experiments were repeated three times. The slopes (a) and intercepts (b), their respective standard errors (s_a) and (s_b) and the coefficient of correlation (r) are shown in Table 2 for HPTLC and Table 3 for HPLC. All the results are summarized in Table 2 for HPTLC and Table 3 for HPLC. Correlation coefficients for the linear fit were excellent, > 0.990.

In HPTLC for each compound, the concentration of about 4.5 mmol l^{-1} was the largest value that could be used which limited the calibration curve.

The limit of detection of different compounds had about the same value in HPLC and in HPTLC respectively, perhaps because of their similar structure.

With both methods described above, the p-hydroxybenzoic esters in drugs were identified and quantified, the first was a foam with a MPHB and PPHB mixture (Fig. 3a and Fig. 4a) and the second was ear drops with only MPHB (Fig. 3b Fig. 4b). The experiments were repeated five times, the coefficient of variation was less than 5% in most cases indicating a good reproducibility and stability for the chromatographic system. The results are summarized in Table 4.

In HPTLC analysis, several samples can be placed on the same plate, 9 samples with a 10×10 cm^2 plate and 19 with a 10×20 cm^2 plate. The analysis time was always about 1 h (10 min for the first migration, 20 min for the second migration, 2×5 min for drying of the plate and 20 min for the scanner). In HPLC, for the same sample number the required time varied from about 72 to 152 min. Therefore, the HPTLC was often faster than HPLC if there were a lot of samples.

4. Conclusion

Even cruder samples can be analyzed in HPTLC because each plate is used only once. The choice of the sample solvent for HPTLC is not as critical because it is evaporated before

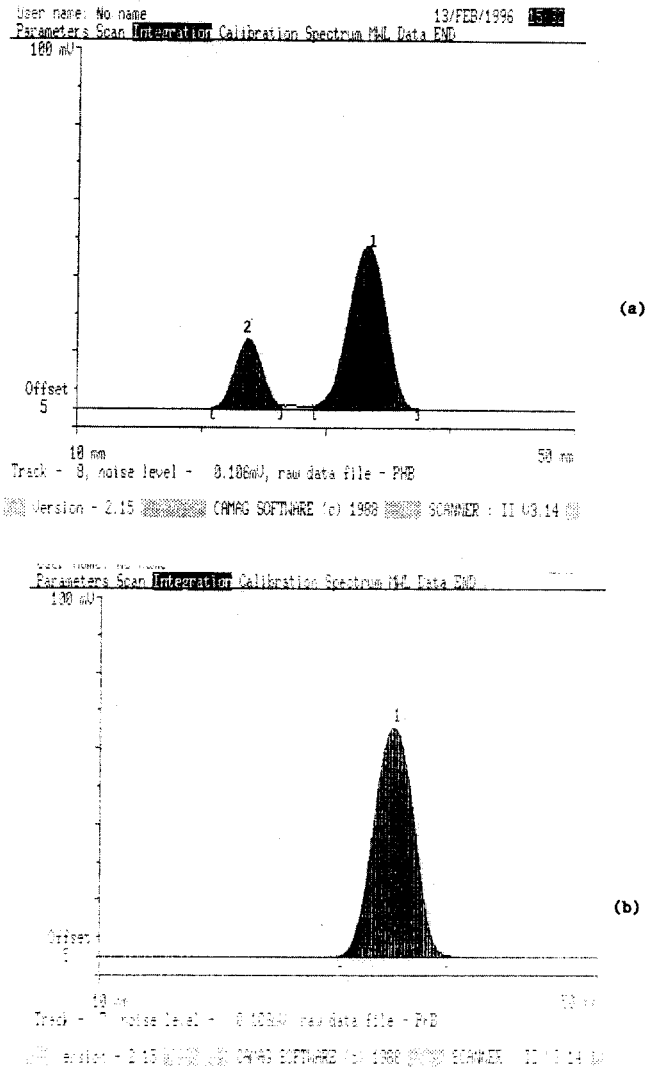

Fig. 3. Chromatograms in HPTLC: (a) foam; and (b) ear drops.

the development of the layer and separates undesirable compounds such as the other constituents of the drugs.

The proposed HPTLC is a quantitative method with precision that is as good as that for HPLC. It is a reproducible, rapid and cheap method and can be used in the routine determination of parabens in drugs when their concentrations are not too great.

(a)

CHROMATOPAC C-R3A FILE 0
SAMPLE NO 0 METHOD 421
REPORT NO 281

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.888	138894			2.033	
2	2.045	5793689	S		84.8027	
3	4.843	899380			13.1643	
	TOTAL	6831962			100	

(b)

CHROMATOPAC C-R3A FILE 0
SAMPLE NO 0 METHOD 421
REPORT NO 287

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	0.878	138915			1.8846	
2	2.015	7232341			98.1154	
	TOTAL	7371256			100	

Fig. 4. Chromatograms in HPLC: (a) foam; and (b) ear drops.

Table 4
Determination of parablens in mg for 100 g of drug using HPLC or HPTLC

	Compound		
	For 100 g of foam		Ear drops
	MPHB mg %	PPHB mg %	MPHB mg %
HPLC	84.1	15.3	146
HPTLC	83.5	14.8	146
Limit value	<120 mg %	<20 mg %	<150 mg %

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