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High-performance liquid chromatographic determination of bisacodyl in pharmaceutical dosage forms marketed in Australia

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Abstract

HPLC methods have been developed for the assay of bisacodyl in various pharmaceutical forms. The extraction procedures are simple and the HPLC conditions separate bisacodyl from its degradation products. The chromatography was performed using a Merck LiChrospher RP-select B column, a mobile phase of 55% acetonitrile/45% 0.05 M KH_2PO_4 and detection by UV at 214 nm.

Keywords: Bisacodyl; Bisacodyl monoester; Pharmaceutical formulations; Reversed-phase high-performance liquid chromatography

1. Introduction

Bisacodyl, 4,4'-(2-pyridylmethylene)di-(phenylacetate), is a stimulant laxative, used for short-term treatment of constipation and for bowel evacuation before investigational procedures or surgery.

The British Pharmacopoeia monograph [1] for bisacodyl suppositories describes a non-aqueous titrimetric assay method, whereas the assay method in the monograph for the enteric-coated tablets [2] is a spectrophotometric analysis by UV using chloroform as the extraction solvent.

The USP monograph [3] for bisacodyl tablets describes an HPLC method with a mobile phase of 0.074 M sodium acetate in water (adjusted to pH 7 with acetic acid) and acetonitrile (55:45), and UV detection at 265 nm. The USP monograph [3] for bisacodyl suppositories includes an assay procedure which utilises these HPLC conditions, but the sample preparation involves repeated two-phase extractions. Also, the mobile phase is unsuitable for detection at the lower wavelengths required to enhance the sensitivity because of the presence of acetate. There is no BP or USP monograph for bisacodyl enema.

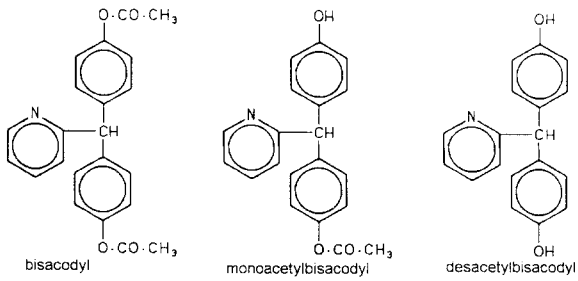

Fig. 1. Structures of bisacodyl, monoacetylbisacodyl and desacetylbisacodyl.

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Several liquid chromatographic determinations of bisacodyl and its degradation products, desacetylbisacodyl and monoacetylbisacodyl (bisacodyl monoester), in biological samples have been published [4–6]. (The structures of these compounds are shown in Fig. 1.) However, only one report is cited for the HPLC determination of bisacodyl and its degradation products in tablets and suppositories [7]. The sample preparation for the suppositories in this procedure required at least three extractions to approach 100% recovery.

The purpose of this project was to develop a set of chromatographic conditions and simple sample preparation procedures for the determination of bisacodyl in all three dosage forms.

2. Experimental

2.1. Materials

Bisacodyl reference obtained from Profarmaco and bisacodyl monoester came from Fisons. The acetonitrile used (Mallinckrodt) was chromAR HPLC grade. The ethanol (CSR) used was 99.8% (high grade). Potassium dihydrogen orthophosphate (BDH) and disodium hydrogen orthophosphate (BDH) were analytical grade. The GF/C glass microfibre filter papers were obtained from Whatman.

The following products were used in developing the bisacodyl assay procedures: 10 mg suppositories (Petrus Pharmaceuticals, Midland, WA, Australia), Durolax 5 mg suppositories and Durolax 5 mg enteric-coated tablets (Boehringer Ingelheim, Artarmon, NSW, Australia), and Bisalax 5 mg enteric-coated tablets and Bisalax 10 mg per 5 ml micro-enema (Fisons Pharmaceuticals, Castle Hill, NSW, Australia).

2.2. Chromatography

The liquid chromatographic system consisted of a Waters 712 WISP, a Waters 510 HPLC pump, a Perkin-Elmer LC55 Spectrophotometer and a Hewlett Packard HP3396A integrator.

The mobile phase consisted of acetonitrile and 0.05 M KH_2PO_4 (55:45). The phosphate buffer was prepared in purified water and filtered through a 0.45 μm filter employing a Millipore filtration assembly prior to mixing with acetonitrile. The resultant mobile phase was degassed by sonication under vacuum immediately prior to use.

Chromatography was carried out on a Merck LiChrospher 60 RP-Select B column (5 μm particle size, 250 mm × 4 mm). The operating conditions were: flow rate, 1.0 ml min^{-1}; injection volume, 20 μl; detection, UV; wavelength, 214 nm; AUFS, 0.25 (tablets and micro-enema), 0.064 (suppositories). Under these conditions, bisacodyl eluted at 7.1 min. A chromatogram of a bisacodyl standard solution is shown in Fig. 2.

3. Preparation of standards and samples

3.1. Suppositories

Standard solution

Approximately 5 mg of bisacodyl was weighed accurately and dissolved in 100 ml ethanol. A 10 ml aliquot of this solution was diluted to 20 ml with mobile phase and filtered through a GF/C glass microfibre paper before injection (final concentration 0.025 mg ml^{-1} bisacodyl).

Sample preparation

10 mg suppositories. One suppository was placed in a 200 ml volumetric flask, 150 ml of ethanol was added and the flask was heated to 45°C until the suppository was completely dissolved in the ethanol. The solution was cooled to room temperature and diluted to volume with ethanol. A 10 ml aliquot of the ethanolic extract was diluted to 20 ml with mobile phase and the solution was filtered through a GF/C glass microfibre paper before injection (final concentration 0.025 mg ml^{-1} bisacodyl).

5 mg suppositories. One suppository was placed in a 100 ml volumetric flask, 75 ml of ethanol was added and the flask was heated to 45°C until the suppository completely dissolved in the ethanol. Procedure was then the same as for the 10 mg suppositories. (The final concentration of bisacodyl was 0.025 mg ml^{-1}). A chromatogram of the suppository sample solution is shown in Fig. 3.

3.2. Tablets

Standard solution

Approximately 5 mg of bisacodyl was weighed accurately and dissolved in 20 ml ethanol.

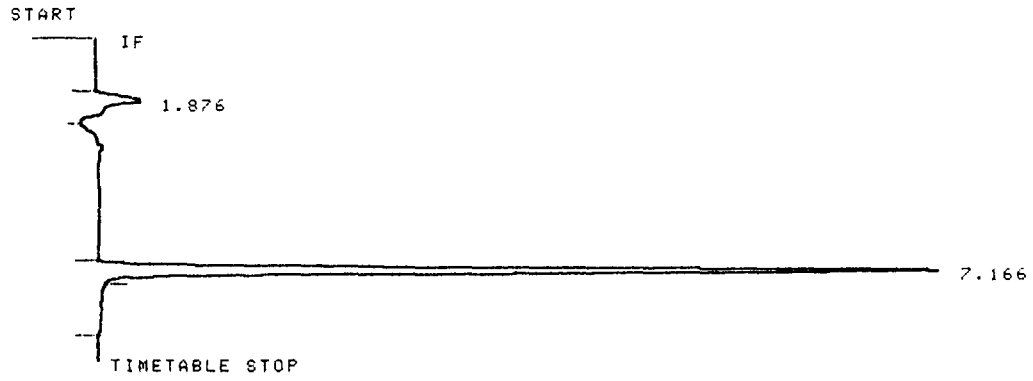


Fig. 2. Chromatogram of bisacodyl standard.

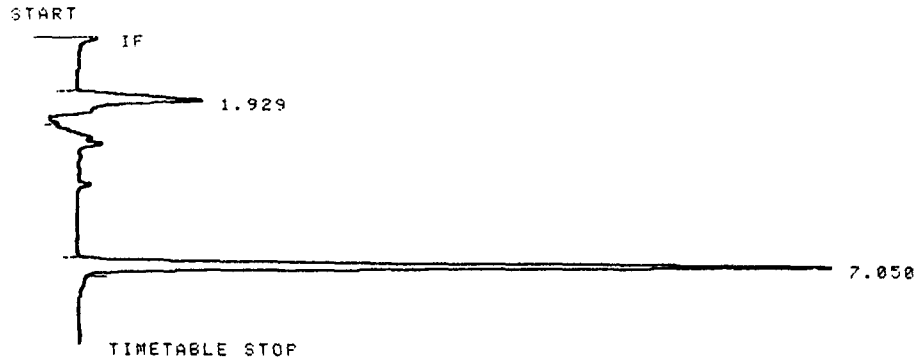


Fig. 3. Chromatogram of Durolax 5 mg suppository sample.

A 10 ml aliquot of this solution was diluted to 20 ml with mobile phase and filtered through a GF/C glass microfibre paper before injection (final concentration 0.125 mg ml^{-1} bisacodyl).

Sample preparation

Ten tablets were placed in a 200 ml volumetric flask, 10 ml mixed phosphate buffer pH 6.8 [1] was added and the flask was shaken for about 15 min (until the tablets were soft). The tablets were then broken using a glass rod and the shaking of the flask was continued until the tablets were completely disintegrated. About 170 ml ethanol was added and the flask was placed in an ultrasonic bath for about 25 min (with occasional shaking). The flask was cooled to room temperature and diluted to 200 ml with ethanol. A 10 ml aliquot of this ethanolic extract was diluted to 20 ml with mobile phase. The solution was filtered through a GF/C glass microfibre paper. (The final concentration of bisacodyl was 0.125 mg ml^{-1}.) Chromatograms of the Durolax and Bisalax tablet sample solutions are shown in Figs. 4 and 5.

[1] Dissolve 2.88 g of disodium hydrogen orthophosphate and 1.145 g of potassium dihydrogen orthophosphate in sufficient water to produce 100 ml.

3.3. Micro-enema

Standard solution

Approximately 5 mg of bisacodyl was weighed accurately and dissolved in 20 ml ethanol. A 10 ml aliquot of this solution was diluted to 20 ml with mobile phase and filtered through a GF/C glass microfibre paper before injection (final concentration 0.125 mg ml^{-1} bisacodyl).

Sample preparation

The unopened sample container was weighed and then shaken mechanically for about 20 min. The contents of the container were transferred into a 50 ml volumetric flask. The empty container was reweighed and the sample weight taken for assay was calculated. About 40 ml of ethanol was added to the flask which was placed in an ultrasonic bath for about 15 min. The flask was then cooled to room temperature and diluted to volume with ethanol. A 10 ml aliquot of this ethanolic extract was diluted to 20 ml with mobile phase. The solution was filtered through a GF/C glass microfibre paper. (The final concentration of bisacodyl was 0.125 mg ml^{-1}.) Fig. 6 is a chromatogram of the Bisalax micro-enema sample solution.

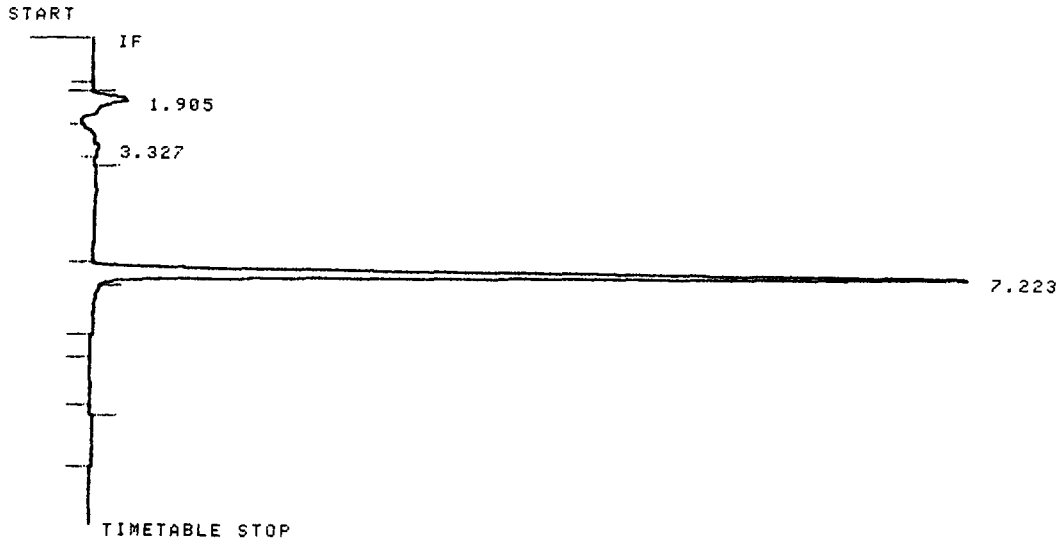


Fig. 4. Chromatogram of Durolax 5 mg tablet sample.

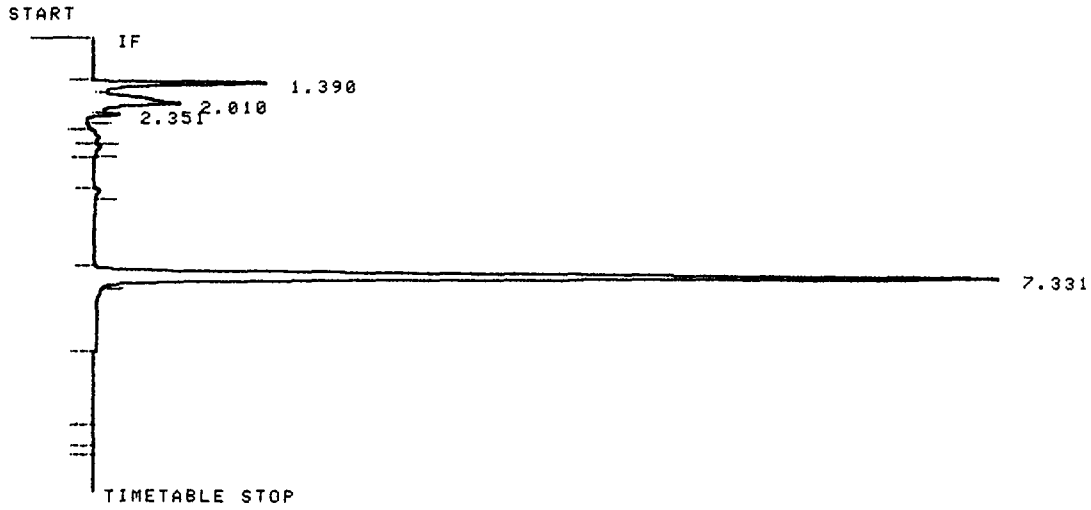


Fig. 5. Chromatogram of Bisalax 5 mg tablet sample.

4. Results and discussion

The proposed methods were assessed for specificity, linearity, precision, accuracy and stability. A summary of the data obtained is shown in Table 1.

4.1. Specificity

Attempts were made to degrade the bisacodyl standard to determine whether the HPLC method was able to separate any degradation products/impurities from the bisacodyl. Solutions of 5 mg bisacodyl in 100 ml of either 0.1 M hydrochloric acid or 70% 0.1 M sodium hydorxide/30% ethanol were prepared. Aliquots of these solutions were further diluted 1:1 with mobile phase and chromatographed immediately. After 24 h, further aliquots of the acidic and alkaline solutions were diluted as before and chromatographed.

Bisacodyl in the alkaline solvent degraded to two compounds immediately, with 68% converting to bisacodyl monoester (RT 4.6 min) and the remainder converting to a compound eluting at 3.3 min, as shown in Fig. 7(a). (Chromatograms of bisacodyl and its degradation products obtained by Salvesan et al. [7] suggest that the compound is desacetylbisacodyl. However, no desacetylbisacodyl was available to allow definite identification of the compound.) After 24 h, total degradation of bisacodyl in the alkaline solution had occurred; there was only one peak in the chromatogram (Fig. 7(b)) at 3.3 min. There was no immediate degradation of bisacodyl in the acidic medium.

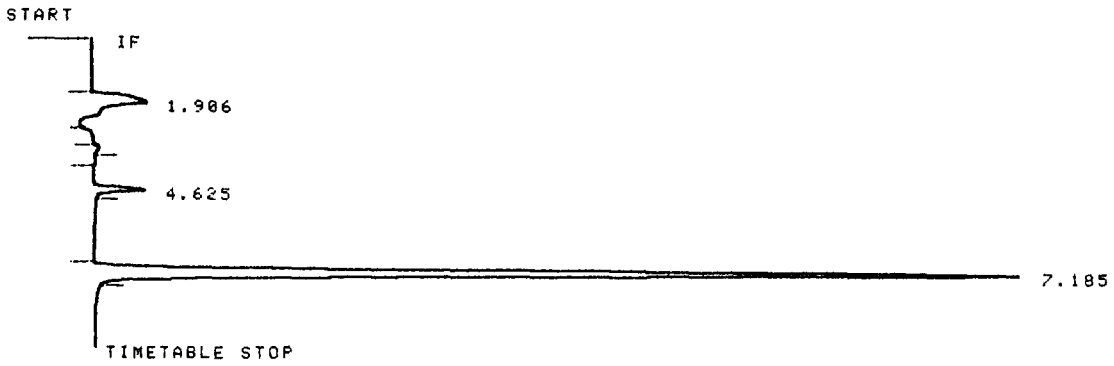


Fig. 6. Chromatogram of Bisalax micro-enema sample showing bisacodyl monoester at 4.6 min.

Table 2
Calibration curve parameters of bisacodyl

	(*n*)	Linear range ($\mu g\ ml^{-1}$)	Regression line equation[a]	Correlation coefficient (*r*)
Suppository	4	11.34–37.82	$y = -10.0511 + 1825.485x$	1.0000
Tablets/enema	4	58.02–194.3	$y = 155.1623 + 1789.703x$	1.0000

[a] y = peak area, x = concentration ($\mu g\ ml^{-1}$).

After 24 h, 35% of the bisacodyl (RT 7.1 min) had degraded to bisacodyl monoester (RT 4.5 min). Figs. 8(a) and 8(b) show the chromatograms obtained.

For each of the products tested, any excipients absorbing at wavelengths from 190 to 350 nm were chromatographed to check for potential interference. In addition, the samples were analysed by changing the mobile phase to 45% CH_3CN/55% 0.05 M KH_2PO_4. The retention time increased from 7 to 14 min. No additional peaks were observed in any of the samples and the assay values agreed with those obtained with the initial (stronger) mobile phase. Thus, the chromatographic conditions separated all excipients from the bisacodyl.

4.2. Linearity

Linearity was checked by chromatographing four standard solutions spanning 50–150% of the expected working concentrations (0.025 mg ml^{-1} for the suppositories, and 0.125 mg ml^{-1} for the tablets and micro-enema). The linearity of the two plots of peak area against concentration was assessed by linear regression analysis. In both cases, the correlation coefficient, *r*, was 1.0000. The calibration curve parameters are shown in Table 2.

4.3. Precision

The system precision was determined by chromatographing six injections of the standard solution, and calculating the relative standard deviation (RSD) of the peak area and retention time (RT) (see Table 1). The method precision was established by chromatographing five or more sample replicates. The calculated RSD values were well within accepted limits.

4.4. Accuracy

The accuracy of the methods was checked by comparing the HPLC assay values with those obtained using an established assay method.

The 10 mg suppositories and the tablets were analysed according to their respective BP monograph methods. The assay value obtained for the 10 mg suppositories by titration (101.2%) was slightly higher than that obtained by HPLC. This small difference can possibly be attributed to the non-specificity of the BP titration method.

In the case of the tablets, the UV results are several percent higher than those obtained by the HPLC method. This is most likely due to the non-specific nature of the UV method.

The micro-enemas were analysed using the method supplied by the manufacturer (Fisons Pharmaceuticals) which uses UV spectrophotometry to quantify the bisacodyl. The assay

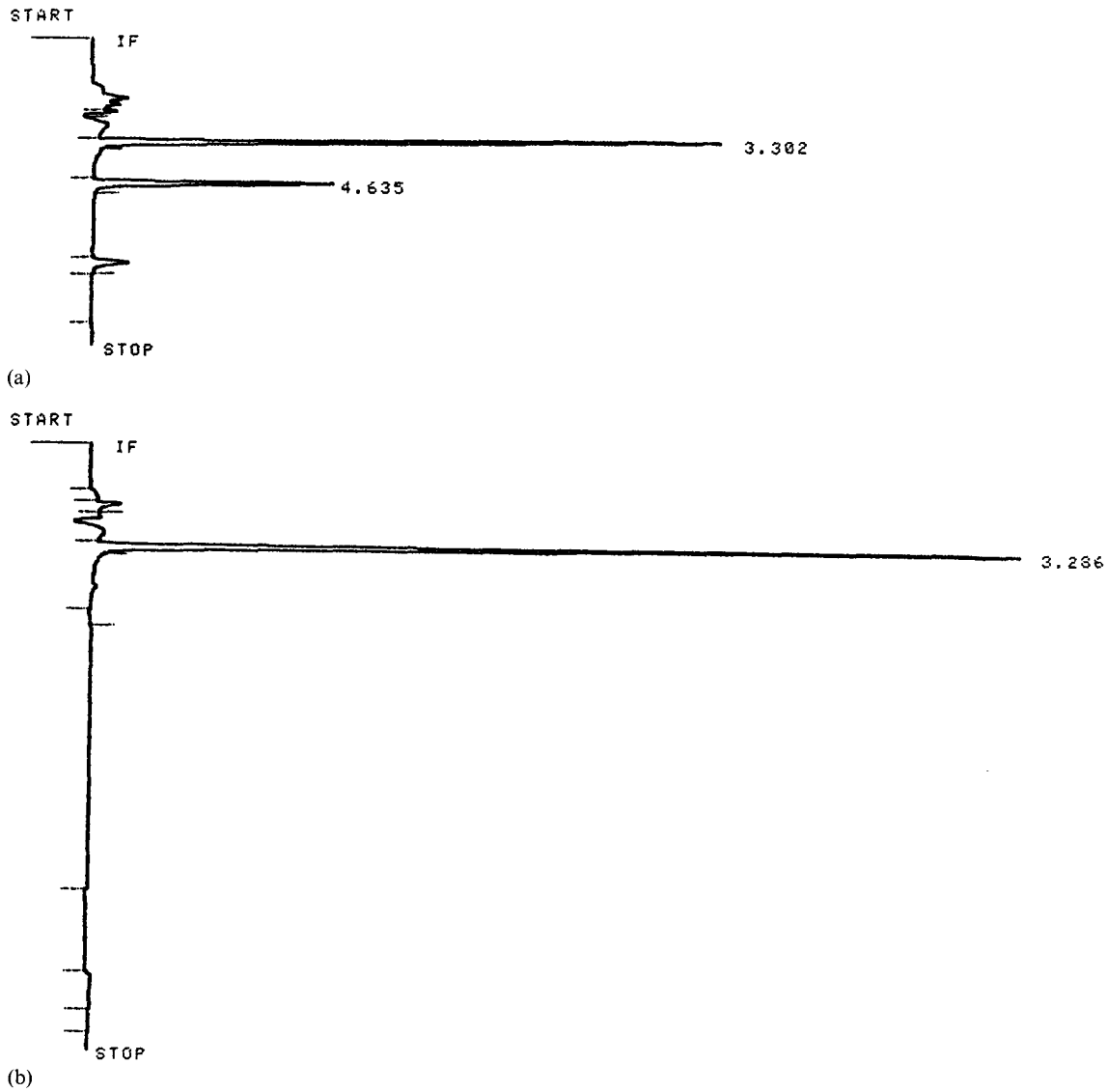


Fig. 7. Chromatograms of bisacodyl in alkaline solution: (a) immediately after preparation of solution; (b) after 24 h.

value obtained was 103.7%. The HPLC method detected 4.5% of bisacodyl monoester, calculated as a percentage of the bisacodyl content of the sample. The UV method of the manufacturer is unable to differentiate between bisacodyl and bisacodyl monoester. Therefore, the HPLC method developed in this study gives a more accurate assay result.

The limit of detection for bisacodyl monoester was about 0.0002 mg ml^{-1}, which equates to 0.2% of the bisacodyl content of the microenema. This is well below the level required for the quality check of the microenema.

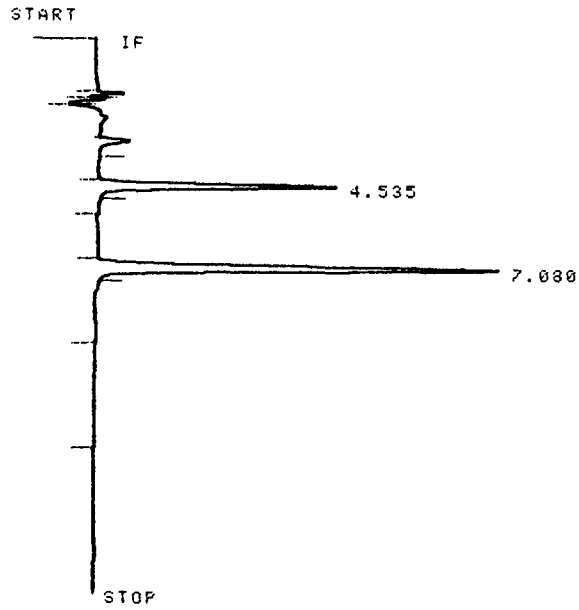
4.5. Recovery

The recovery of the HPLC method was checked by adding known amounts of bisacodyl to each of the different dosage forms (see Table 3). The calculated recoveries were satisfactory.

4.6. Stability

The stability of the ethanolic sample solutions at 20°C 24 h after preparation was ver-

(a)



(b)

Fig. 8. Chromatograms of bisacodyl in acidic solution: (a) immediately after preparation of solution; (b) after 24 h.

Table 1
Method validation data

Product	Precision System (% RSD)		Precision method (% RSD)	% Recovery (standard) additions)	Accuracy	
	Peak area	RT			HPLC (% Stated amount)	Alternative
Suppositories (10 mg) (Petrus Pharmaceuticals)	0.15	0.06	0.36 ($n = 5$)	-	98.0% ($n = 5$)	101.2% ($n = 2$)
Suppositories (5 mg)						
Bisalax	0.13	0.13	0.95 ($n = 6$)	102.7%	100.8% ($n = 6$)	
Tablets						
Durolax (5 mg)	0.31	0.38	0.86 ($n = 6$)	102.0%	97.3% ($n = 6$)	104.7% ($n = 4$)
Bisalax	0.39	0.17	1.01 ($n = 6$)	98.7%	99.3% ($n = 6$)	103.2% ($n = 2$)
Micro-enema (10 mg 5 ml^{-1})						
Bisalax	0.14	0.14	0.39 ($n = 10$)	100.6%	95.2% ($n = 10$)	103.7% ($n = 2$)

Table 3
Recoveries from different dosage forms

Product	Bisacodyl from sample ($\mu g\ ml^{-1}$)	Added bisacodyl ($\mu g\ ml^{-1}$)	% Recovery
Suppositories (5 mg)			
Bisalax	12.5	7.08	103.8
	12.5	14.15	102.1
	12.5	24.77	102.1
Tables (5 mg)			
Durolax	62.5	37.0	101.7
	62.5	74.0	101.8
	62.5	129.6	102.4
Bisalax	62.5	37.0	98.2
	62.5	74.0	98.4
	62.5	129.6	99.4
Micro-enema ($10\ mg\ ml^{-1}$)			
Bisalax	66.6	37.7	100.9
	66.6	75.4	101.0
	66.6	132.0	99.8

ified by re-assaying them (after dilution with mobile phase). There was no indication of any decomposition of bisacodyl in the samples.

5. Conclusion

The reversed-phase HPLC method developed in this study is specific, and capable of separating bisacodyl from its degradation products and excipients. The extraction of bisacodyl from various dosage forms is simple and bisacodyl is stable in the extraction solvent.

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