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Analysis of a complex analgesic formulation by high-performance liquid chromatography with column-switching

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Abstract: An isocratic method is described which allows the complete separation of acetylsalicylic acid, paracetamol, caffeine, carbromal, bromisoval and codeine as well as the potential impurities salicylic acid, diacetyl-*p*-aminophenol and acetylcodeine. A column-switching technique was developed employing two columns (10 cm and 25 cm) containing 7-μm Zorbax C_8 material. The separation is better and faster than that obtained with a gradient elution method.

Keywords: *Reversed-phase high-performance liquid chromatography; column-switching; acetylsalicylic acid; analgesic tablet formulation.*

Introduction

During the past decade the value of high-performance liquid chromatography (HPLC) has been frequently demonstrated for the analysis of multicomponent analgesic formulations [1–14]. Most formulations contain acetylsalicylic acid which not only forms salicylic acid by decomposition, but also interacts with other components of the formulation by acetylation. Examples of acetylation discussed in the literature include paracetamol [14–17], phenylephrine [13, 18], codeine [19, 20], ascorbic acid [13] and homatropine [21].

In this paper an HPLC method is described which permits the quantitative determination of all the components of an analgesic tablet containing acetylsalicylic acid, paracetamol, caffeine, carbromal, bromisoval and codeine. The method also separates potential impurities such as salicylic acid, acetylcodeine and diacetyl-*p*-aminophenol. The nine substances are separated isocratically by the combined use of a 10 cm and a 25 cm reversed-phase C_8 column using a column-switching technique. The results are compared with those obtained on a 25 cm column using a gradient elution system.

Experimental

Samples

Five batches of normally-aged tablets (Perdolan®, Janssen Pharmaceutica, Beerse, Belgium) were available. At the time of analysis they were 1, 2, 3, 4 and 5 years old

respectively. Besides the active components, i.e. acetylsalicylic acid (200 mg), paracetamol (200 mg), anhydrous caffeine (50 mg), carbromal (30 mg), bromisoval (10 mg) and codeine phosphate hemihydrate (10 mg), the tablets contained maize starch, microcrystalline cellulose, povidone, liquid paraffin and talc.

Reagents and chemicals

Reference substances for the active components were kindly donated by the manufacturer of the tablets. All these reference substances and salicylic acid were of pharmacopoeial grade. Diacetyl-*p*-aminophenol [15, 22] and acetylcodeine [20] were prepared by procedures described in the literature. *N*-(4-Isopropoxyphenyl) acetamide, used as the internal standard, was prepared by heating paracetamol (0.1 mol), 1-bromopropane (0.1 mol) and potassium hydroxide (0.1 mol) in 75 ml of ethanol on a waterbath for 3 h. Crystallization was achieved by the addition of 0.15 l of water and cooling. The substance was purified by further crystallization from ethanol–water and dried at 80°C. The yield was about 80%, and the melting point 130–131°C. The internal standard solution contained 0.25% m/v in methanol. Methanol for extraction (99+%, Janssen Chimica, Beerse, Belgium) was distilled before use. Water was distilled twice. Organic solvents for HPLC were obtained from Rathburn (Walkerburn, Scotland). Tetramethylammonium hydroxide 20% m/m in methanol was from Janssen Chimica. Other reagents were of *pro analysi* quality (E. Merck, Darmstadt, FRG).

Columns

Columns containing 7-μm Zorbax C_8 (Du Pont, Wilmington, DE, USA) were freshly packed in the laboratory. A column was attached to a 10 cm pre-column and both were filled with carbon tetrachloride before being attached to the slurry reservoir (25 cm × 0.5 in. o.d. stainless steel tubing). The packing material, 4.0 g for a 25 cm × 4.6 mm column and 1.7 g for a 10 cm × 4.6 mm column, was slurried in 15 and 9 ml respectively, of carbon tetrachloride. The slurry was sonicated for 4 min and quickly introduced into the slurry reservoir. The slurry was immediately packed into the column at about 600 bar, using a Haskel pump Model DSTV-122 (Haskel, Burbank, CA, USA), with an inlet pressure of 5 bar and with methanol–water (80:20, v/v) as the pressurizing liquid. The other columns were the same as those used in another study [23].

Apparatus and operating conditions

The pump unit for isocratic work consisted of a Milton Roy minipump (Laboratory Data Control, Riviera Beach, FL, USA) equipped with a flexible hose, Model SS-4HO-6S4 (Swagelok, Crawford fitting, Solon, OH, USA) used as a pulse dampener [24] and a Bourdon pressure gauge (Covena, Brussels, Belgium). A 20-μl loop injector Model CV-6-UHPa-N60 (Valco, Houston, TX, USA), a Pye Unicam LC 3 UV-detector (Pye Unicam, Cambridge, UK) set at 225 nm and at 0.04 a.u.f.s. and a recording integrator Model 3390 A (Hewlett Packard, Avondale, PA, USA) were used. For isocratic elution the columns were assembled as shown in Fig. 1. The 10 and 25 cm columns were connected through a Valco Model CV-6-UHPa-N60 injector, used as the column-switching valve. At the moment of injection this valve was in position 1, after 7 min it was switched to position 2 and after 41 min it was switched back to position 1. The mobile phase for isocratic elution consisted of methanol (270 ml)–1 M phosphoric acid (35 ml)–20% m/m tetramethylammonium hydroxide solution in methanol (1.00 g)–water (up to 1000 ml).

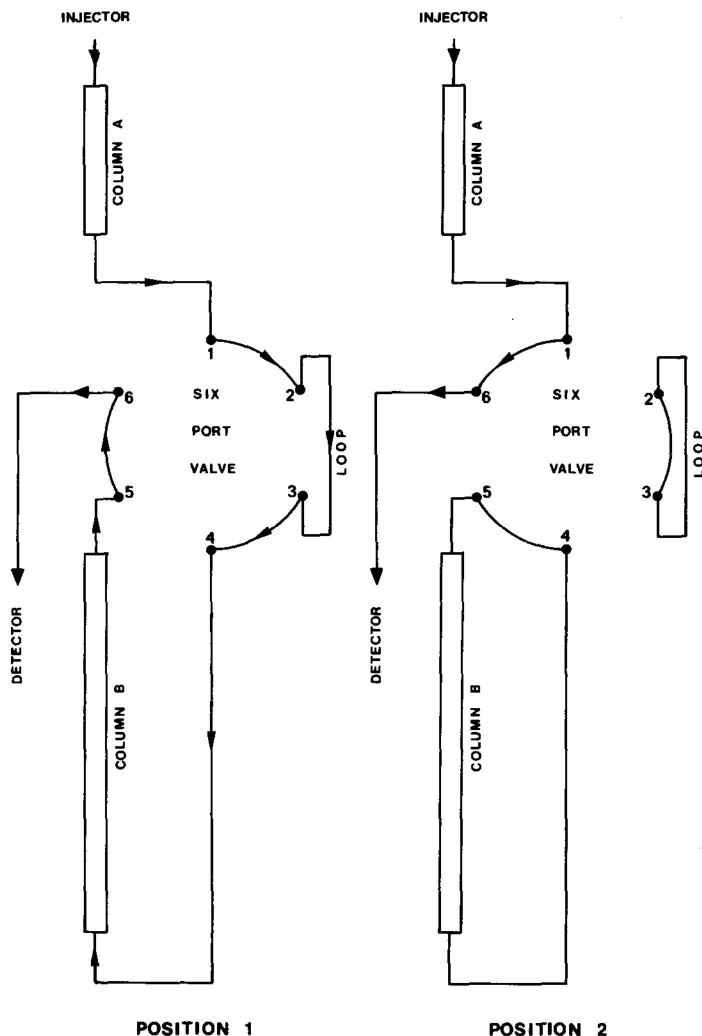

Figure 1
Equipment for isocratic elution with column-switching. Column A: 10 cm × 4.6 mm, column B: 25 cm × 4.6 mm. Both columns are packed with 7-μm Zorbax C_8.

For gradient elution a Varian Model LC 4200 chromatograph (Varian, Palo Alto, CA, USA) was used with the 25 cm column only. Pump A and pump B contained methanol (A: 100 ml; B: 500 ml)–1 M phosphoric acid (A and B: 35 ml)–20% m/m tetramethylammonium hydroxide solution (A and B: 1.90 g)–water (up to 1000 ml). The gradient profile adopted was $t = 0$, $\%B = 20$; 39 min, 20%; 44 min, 60%; 60 min, 60%. The original mobile phase was then reset through a rapid decreasing gradient. The flow rate was 1.0 ml min^{-1}. The columns were maintained at 30°C by a glass water jacket connected to a circulating water bath [25].

Preparation of the sample solutions

Ten tablets were weighed and homogenized by milling for two periods of 15 s. An aliquot corresponding to half of a tablet was transferred to a 50 ml volumetric flask, then

30 ml methanol, 0.5 ml formic acid and 5.0 ml internal standard solution were added. The flask was sonicated for 3 min and the mixture diluted to 50.0 ml with methanol. The flask was sonicated for 30 s and the mixture was filtered through paper. The first few milliliters were discarded. An aliquot of the filtrate was passed through a membrane filter (1.2 μm), and the filtrate injected immediately.

Isocratic chromatographic analysis

Calibration curves (y = peak area ratio, x = mg substance/100 ml of the solution injected, r = correlation coefficient, $S_{y,x}$ = standard error of estimate) were obtained with reference substance solutions containing acetylsalicylic acid ($y = 0.0559x + 0.548$, $r = 0.9999$, $S_{y,x} = 0.017$), paracetamol ($y = 0.0315x + 3.179$, $r = 0.9953$, $S_{y,x} = 0.054$), caffeine ($y = 0.0542x - 0.040$, $r = 0.9996$, $S_{y,x} = 0.011$), carbromal ($y = 0.0103x - 0.020$, $r = 0.9997$, $S_{y,x} = 0.001$), bromisoval (y $0.0127x + 0.011$, $r = 0.9946$, $S_{y,x} = 0.002$), codeine phosphate ($y = 0.0410x - 0.018$, $r = 0.9984$, $S_{y,x} = 0.004$), salicylic acid ($y = 0.0499x + 0.002$, $r = 0.9993$, $S_{y,x} = 0.001$) diacetyl-*p*-aminophenol ($y = 0.0466x$, $r = 0.9999$, $S_{y,x} = 0.004$) and acetylcodeine ($y = 0.0201x$, $r = 0.9969$, $S_{y,x} = 0.003$). The solutions of the active components used in these experiments were prepared to cover the range of 80 to 120% of the theoretical values. The solutions of the degradation products covered the range up to 5%.

The precision of the method was checked by quadruple analysis of a solution containing known concentrations of the active components, acetylcodeine (5% relative to codeine) and diacetyl-*p*-aminophenol (0.25% relative to paracetamol). Salicylic acid was not included since it could also have been formed upon storage of the solution. The percentage found and the relative standard deviation (RSD) were: acetylsalicylic acid (100.5, 0.6), paracetamol (100.2, 0.8), caffeine (100.4, 0.6), carbromal (101.1, 0.5), bromisoval (99.1, 1.7), codeine phosphate (100.4, 1.5), acetylcodeine (105.5, 6), diacetyl-*p*-aminophenol (104.5, 3.8).

Results and Discussion

Chromatographic method

In the search for an isocratic system, a number of C_8 and C_{18} reversed-phase columns (25 cm × 4.6 mm) were compared using mobile phases containing methanol–1 M phosphoric acid–water, x:5:(95 − x). Differences in resolution were observed and even differences in the sequence of elution, as shown in Table 1. The methanol content was adjusted to obtain comparable retention times. In our laboratory, such important differences in the order of elution were also observed for cephalosporins [23]. Codeine, which is protonated in this system, shows the highest retention on RSiL C_{18} LL (LL stands for low loading). This suggests interaction with the silanol groups of the underlying silica gel. This phenomenon was also observed previously on RSiL C_{18} LL with the positively charged cephaloridine [23]. Paracetamol elutes first on all the columns and carbromal usually elutes last. From the retention times it was considered that an isocratic method with one column would not permit the separation of all the compounds within a reasonable time. Results obtained with the Zorbax column are shown in Fig. 2a. When this column was used with other organic modifiers such as acetonitrile, tetrahydrofuran or propan-2-ol no major improvements in selectivity were obtained. Variation of the pH ranging from 2 to 4.5 influenced the retention times of acetylsalicylic acid and salicylic acid only.

Table 1
Elution order on different stationary phases

Stationary phase		Paracetamol	Caffeine	Codeine	Acetylsalicylic acid	Bromisoval	Carbromal
Zorbax BP-C_8	7-µm	1	2	3	4	5	6
Nucleosil C_8	10-µm	1	5	2	3	4	6
Polygosil C_8	10-µm	1	5	2	4	3	6
LiChrosorb C_8	10-µm	1	2	3	4	5	6
µ Bondapak C_{18}	10-µm	1	3	2	4	5	6
Nucleosil C_{18}	10-µm	1	3	2	4	5	6
LiChrosorb C_{18}	10-µm	1	3	2	4	5	6
Partisil ODS	10-µm	1	6	2	4	3	5
RSil C_{18} LL	10-µm	1	4	5	2	3	6

Mobile phase: methanol–1 M phosphoric acid–water x:5:(95 − x).
The methanol content was adjusted to obtain comparable retention times.

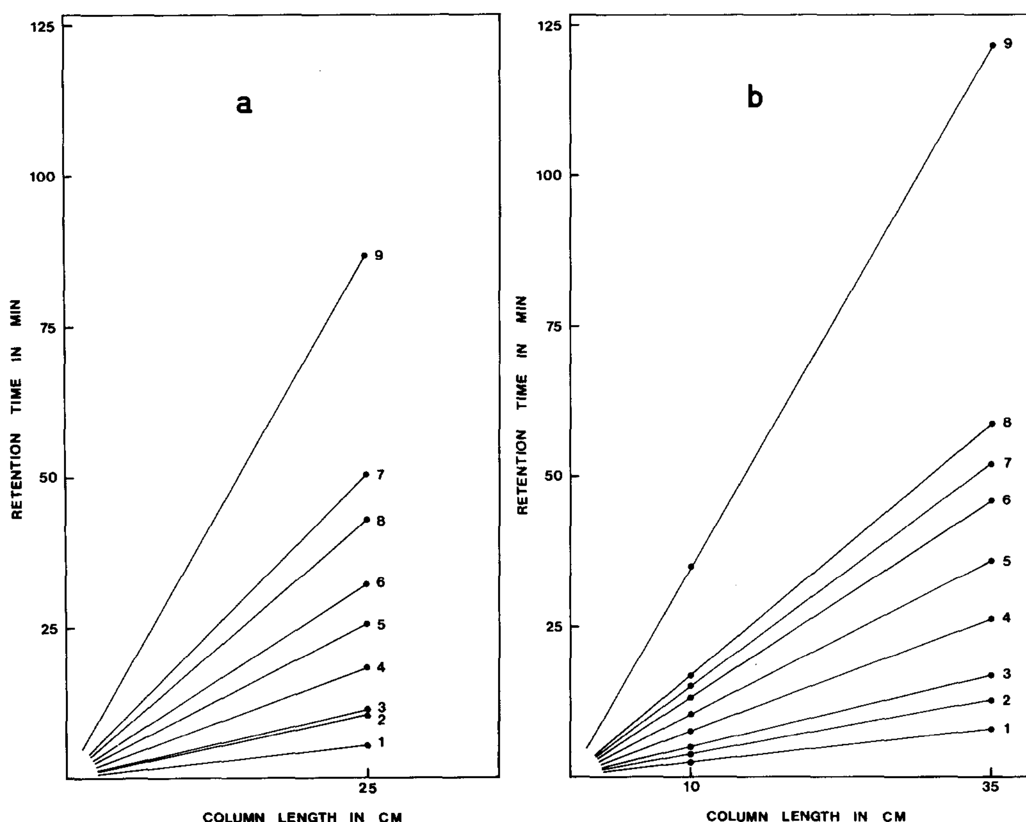

Figure 2
Retention times obtained with Zorbax columns. The retention times are extrapolated for zero column length. (a) Results obtained on a 25 cm × 4.6 mm column with methanol (270 ml)–1 M phosphoric acid (50 ml)–water (up to 1000 ml) as the mobile phase. (b) Results obtained on a 10 cm × 4.6 mm column alone or in combination with a 25 cm × 4.6 mm column with methanol (270 ml)–1 M phosphoric acid (35 ml)–20% m/m tetramethylammonium hydroxide solution in methanol (1.00 g)–water (up to 1000 ml). ● = experimental point. Key: 1, paracetamol; 2, codeine; 3, caffeine; 4, diacetyl-*p*-aminophenol; 5, acetylsalicylic acid; 6, bromisoval; 7, acetylcodeine; 8, salicylic acid; 9, carbromal.

The more usual way of separating such a complex mixture is by using gradient elution. Since this requires more sophisticated equipment, it was decided to solve the problem by an isocratic column-switching technique. In the above-mentioned experiments it was observed that on Zorbax C_8 (Fig. 2a) a good distribution of retention was obtained for the six active components as well as for the three potential decomposition products. This column was therefore used in further experiments. Since retention time increases with column length then for column length zero the retention time also approaches zero, and this simple relationship between retention time and column length was obtained from one experiment on a 25 cm column as shown in Fig. 2a. The figure shows that the nine products can be divided in two groups: a fast eluting group (1–3) of paracetamol, caffeine and codeine which are not sufficiently separated on a 25 cm column and a group of the other six substances which would probably be sufficiently separated on a column of about 10 cm. Therefore it was decided to use a 10 cm and a 25 cm Zorbax C_8 column, connected as shown in Fig. 1. The 6-port valve in this figure could be replaced by a 4-port

valve. The second group would be sufficiently separated on the short column while the separation of the first group could be improved since the total column length is increased to 35 cm.

Two problems still remained to be solved. It was obvious that even on a 35 cm column caffeine and codeine were not sufficiently separated. Furthermore the retention times of the first group had to be adapted by modifying the methanol content of the mobile phase so that the first group was trapped on the 25 cm column while the second group eluted from the 10 cm column to the detector (position 2 in Fig. 1).

Since codeine was protonated in the acid mobile phase its retention was supposed to be determined at least partly by interaction with the silica gel backbone of the stationary phase. This interaction was also demonstrated by the peak shapes of codeine and acetylcodeine which were very broad and asymmetric. The peak shape of basic substances in acid mobile phases may be improved by the addition of quaternary ammonium ions such as tetramethylammonium [26–30]. The addition of tetramethylammonium phosphate solution not only improved the peak shape significantly but also decreased the retention time of codeine and acetylcodeine. Both effects can be explained by competition for the active sites on the silica gel. As shown in Fig. 2b, all the substances were separated on the 35 cm column. It was also checked experimentally that the second group of substances was sufficiently separated on the 10 cm column.

The phosphoric acid content of the mobile phase was not critical. At lower temperature bromisoval and acetylsalicylic acid were less separated. A wavelength of 225 nm was chosen to optimize the detection of bromisoval, carbromal and codeine. When after injection with the column selection valve in position 1 (see Fig. 1), this valve was brought to position 2 after 7 min, paracetamol, codeine and caffeine were trapped on the 25 cm column. The second group with the less polar substances now eluted first from the 10 cm column to the detector. After elution of carbromal (41 min) the column selection valve was again turned to position 1 to elute the polar substances from the 25 cm column.

A chromatogram obtained with an extract spiked with impurities is shown in Fig. 3a. Complete analysis takes less than 1 h.

For qualitative comparison Fig. 3b shows a chromatogram obtained with an extract spiked with impurities using a one-step gradient system as described in the Experimental section. It is clear that the gradient system not only requires more sophisticated equipment but that it also takes more time, i.e. 70 min plus 10 min additional equilibration time against 60 min. The equilibration time is necessary in order to obtain reproducible results. The separation between acetylsalicylic acid and bromisoval was also better with the column-switching method. A disadvantage of this isocratic method is that its development required more time than that for the gradient system, although since the method was to be used for routine analysis this time was justified.

Tablet analysis

Methanol acidified with formic acid was employed to ensure complete extraction of acetylsalicylic acid and salicylic acid [31]. Water was not added to the extraction liquid since it reduces the solubility of paracetamol and enhances hydrolysis of acetylsalicylic acid. The stability of the extract was checked, and the salicylic acid content was found to increase from 0.06% at time zero to 0.49% after 21 h. The influence of the extraction time in the ultrasonic bath was also checked. Care was taken that the bath temperature did not exceed the room temperature. No significant differences were observed for extraction times between 1 and 6 min. An intermediate extraction time of 3 min was

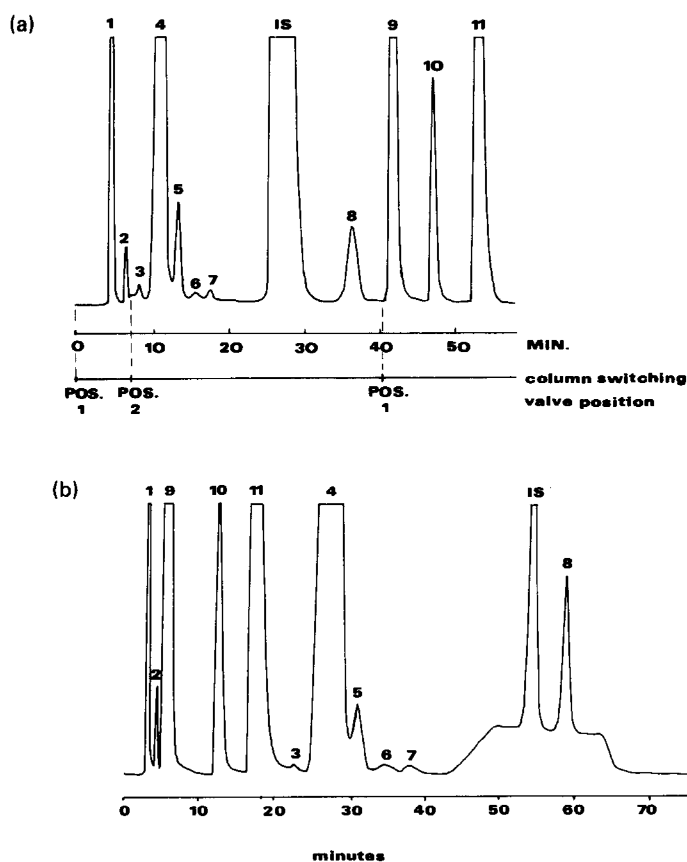

Figure 3
HPLC of a tablet extract spiked with potential degradation products. (a) Isocratic elution with column-switching. (b) Gradient elution. Mobile phases as described in experimental. Key: IS, internal standard; 1, formic acid; 2, methylformate; 3, diacetyl-*p*-aminophenol; 4, acetylsalicylic acid; 5, bromisoval; 6, acetylcodeine; 7, salicylic acid; 8, carbromal; 9, paracetamol; 10, codeine; 11, caffeine.

Table 2
Results obtained with the isocratic method using column-switching

Age of tablets (years)	1	2	3	4	5
Number of analyses	5	4	3	3	3
Acetylsalicylic acid	98.7 (0.5)	98.7 (0.7)	99.6 (0.1)	97.1 (0.6)	95.9 (0.4)
Paracetamol	98.0 (1.0)	95.3 (1.9)	95.7 (1.0)	95.5 (0.7)	96.8 (0.4)
Caffeine	95.7 (0.4)	95.7 (1.5)	93.7 (0.9)	93.9 (0.7)	95.0 (1.0)
Carbromal	97.1 (1.9)	100.5 (1.4)	96.1 (0.7)	98.4 (1.8)	97.4 (0.8)
Bromisoval	93.8 (0.9)	98.6 (2.3)	96.1 (1.7)	93.1 (2.3)	94.4 (1.5)
Codeine phosphate	105.2 (0.6)	108.9 (0.8)	102.3 (0.5)	103.4 (1.4)	103.6 (1.9)
Salicylic acid*	0.10 (8.1)	0.37 (1.6)	0.93 (0.7)	1.27 (0.6)	0.63 (3.3)

Values are reported as percentages of the label contents. RSD values are given in parentheses.
*Calculated as acetylsalicylic acid and reported as percentages of the label content of acetylsalicylic acid.

chosen. Below 1 min the extraction was incomplete and above 6 min the salicylic acid content increased due to hydrolysis of acetylsalicylic acid.

Results obtained for the tablets are compiled in Table 2. The percentage of the label claim is reported. Salicylic acid is calculated as acetylsalicylic acid and contents are reported as percentages of acetylsalicylic acid. Except for codeine phosphate the contents were generally lower than expected. The validity of the calibration curves was checked daily by injection of standards. There is no plausible explanation for why codeine phosphate contents exceeded 100% for all the batches while the contents for all the other active components were always lower. The salicylic acid content increased with time. The fact that the 5-year old batch contained less salicylic acid than the 3- and 4-year old batches may be due to differences in manufacturing quality. The BP 1980 (addendum 1983) [32] limit of 0.6% prescribed for aspirin and codeine tablets was exceeded after 2 years storage but the USP XXI [33] limit of 3.0%, prescribed for most combined aspirin tablets, was not exceeded by any samples.

The relative standard deviation (RSD) was largest for the small peaks. The low RSD values obtained for the active components are an indication of the reproducibility of the extraction. Diacetyl-*p*-aminophenol and acetylcodeine were never observed, although detection limits were 0.05% and 1% respectively. Acetylcodeine was reported to be formed only at higher temperatures and in the presence of moisture [19, 20]. Small quantities of diacetyl-*p*-aminophenol, however, were observed in our laboratory in an analgesic mixture containing acetylsalicylic acid and paracetamol [14]. The formation of diacetyl-*p*-aminophenol by transacetylation has been described to be dependent upon the identity of the other active components and excipients and also upon the conditions of preparation, such as humidity [15–17]. Obviously the conditions for transacetylation were not fulfilled in this formulation.

Results obtained with the isocratic method employing column-switching and with the one-step gradient method are compared in Table 3. The analyses were performed on the 1-year old batch. There is good agreement between the isocratic and gradient results. The results reported for the isocratic method include those already mentioned in Table 2. The RSD for acetylsalicylic acid and bromisoval were higher for the gradient system

Table 3
Comparison between results obtained with the isocratic and with the gradient method

	Isocratic	Gradient
Number of analyses	14	5
Acetylsalicylic acid	99.0 (0.3)	97.8 (1.2)
Paracetamol	97.3 (0.8)	97.5 (0.7)
Caffeine	93.2 (2.4)	90.2 (0.6)
Carbromal	96.4 (0.7)	93.9 (0.6)
Bromisoval	93.3 (1.9)	96.4 (5.2)
Codeine phosphate	102.8 (2.0)	105.9 (1.8)
Salicylic acid*	0.13 (23)	0.13 (28)

Values are reported as percentages of the label contents. RSD values are given in parentheses.

*Calculated as acetylsalicylic acid and reported as percentages of the label content of acetylsalicylic acid.

since the corresponding peaks were not resolved so well. The codeine phosphate content again exceeded 100% whilst the other contents were rather low.

It can be concluded that for the analysis of this complex analgesic formulation the isocratic method with column-switching, using the less sophisticated equipment, is faster and also gives a better separation than a one-step gradient system. In routine analysis this isocratic column switching-method can be a valuable alternative to gradient elution.

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