Journal of Chromatography, 465 (1989) 402–409
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

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Note

Reversed-phase ion-pair liquid chromatographic separation of weak analgesics and related drugs

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(First received November 30th, 1987; revised manuscript received December 2nd, 1988)

Many freely available popular drugs contain one or several pain relievers such as paracetamol, acetylsalicylic acid, codeine, aminopyrine, phenacetin and salicylamide in combination with various other drugs, including hypnotics (carbromal, bromural), stimulants (caffeine), sedatives (barbiturates) and spasmolytics (tiemonium iodide, hyoscyamine). Several commercially available pharmaceuticals combine no less than six active ingredients. The analysis of such mixtures by high-performance liquid chromatography (HPLC) involves serious separation problems as basic, acidic and quasi-neutral compounds with widely different lipophilic properties are involved (Table I). In addition, different UV absorption characteristics and widely varying

TABLE I

COMPOUNDS STUDIED AND pK_a VALUES

pK_a values taken from refs. 10 and 11.

Compound	*Abbreviation*	pK_a
Acetylsalicylic acid	ASA	3.5
Paracetamol	PAR	9.5
Codeine phosphate	COD	8.2
Caffeine	CAF	1
Carbromal	CAR	–
Bromural	BRO	10.8
Aminopyrine	AMI	5.0
Phenacetin	PHE	2.2
Salicylamide	SAA	8.2
Phenobarbital	PHO	7.4
Hyoscyamine	HYO	9.7
Dipyrone	DIP	–
Salicylic acid	SAL	3.0
Tiemonium iodide	TIE	–

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0021-9673/89/$03.50

concentrations of the ingredients in multi-component pharmaceuticals further complicate the analysis.

To our knowledge, no method capable of separating all of the commonly used drugs in commercially available weak analgesic multi-component pharmaceuticals has been reported, although the HPLC analysis of weak analgesics is well documented[1–5]. However, these papers cited deal with the separation of only a few drugs and none of the separation methods used was applicable to more complex analgesic formulations.

Das Gupta[6] reported an excellent separation of a standard mixture of ASA, PAR, CAF, COD, PHE and SAA by reversed-phase (RP) HPLC. Unfortunately, similar commercial drug mixtures containing also CAR and BRO or spasmolytics of the quaternary ammonium type could not be separated unless excessive retention times and/or severe peak tailing were accepted. Cockaerts *et al.*[7] achieved both gradient and isocratic separations of ASA, PAR, BRO, CAR, CAF and COD. The isocratic method gave a good separation of all active components of the pharmaceutical dosage form and even allowed the separation of potential impurities such as SAL, acetylcodeine and diacetyl-*p*-aminophenol. However, the use of a column-switching technique involving two analytical columns and the long analysis time of about 1 h make the method unsuitable for routine analysis.

This paper reports a method for the isocratic liquid chromatographic separation and determination of weak analgesics and related drugs in complex pharmaceutical mixtures, based on the simultaneous use of an anionic and a cationic modifier in the mobile phase[8,9].

EXPERIMENTAL

Chemicals and solvents

All reference drugs were of pharmacopoeial grade (Table I). Sodium chloride (UCB, Belgium) and 85% orthophosphoric acid (Merck, F.R.G.) were of analytical-reagent grade.

N,N-Dimethyloctylamine (DMOA) was obtained from Aldrich (Milwaukee, WI, U.S.A.), anhydrous sodium 1-octanesulphonate (SOS) from Janssen (Belgium) and analytical-reagent grade methanol from UCB. Water was purified by ion-exchange chromatography and subsequent distillation. All other chemicals used were of analytical-reagent grade.

HPLC equipment

Chromatography was performed on an SP 8000 liquid chromatograph (Spectra-Physics, Darmstadt, F.R.G.) equipped with a Model 770 variable-wavelength detector (Spectra-Physics) and a BD 8 single-channel recorder (Kipp & Zonen, Delft, The Netherlands).

Chromatographic conditions

The mobile phase was prepared by dissolving the required amounts of SOS and DMOA in *ca.* 990 ml of methanol–water in various proportions. The mixture was adjusted to pH 3.0 with orthophosphoric acid and diluted to exactly 1000 ml. Before chromatography, the mobile phase was filtered through a 5-μm filter and degassed with helium.

The flow-rate, column temperature, detection wavelength and injection volume were 1 ml/min, 25°C, 220 nm and 10 μl, respectively.

The following columns were used: RSil C_{18}, 5 μm (150 × 4.1 mm I.D.) and RSil C_{18}, 10 μm (250 × 4.6 mm I.D.) from Alltech-RSL (Eke, Belgium) and LiChrosorb RP-18, 5 μm (150 × 4.6 mm I.D.), RP-18, 10 μm (250 × 4.6 mm I.D.) and RP-8, 10 μm (250 × 4.6 mm I.D.) from Chrompack (Middelburg, The Netherlands).

RESULTS AND DISCUSSION

Chromatography on RSil C_{18}

Considerable efforts were made to separate isocratically all active ingredients of Perdolan® dosage forms, as this pharmaceutical is among the most complex in a long series of similar commercial products. The tablets combine ASA, PAR, COD, CAF, BRO and CAR; PHO was also included in the test mixture as this compound is extensively administered in combination with the other drugs. SAL was considered to be the only potential degradation product as a previous study confirmed that acetylcodeine and diacetyl-*p*-aminophenol were not formed in such mixtures under normal conditions of storage over a period of 5 years[7].

The effect of mobile phase composition (*i.e.*, the volume fraction of water, φ, the DMOA and SOS concentrations and the pH) on the capacity factors and selectivities was investigated. The results are shown in Fig. 1. SAL, the main degradation

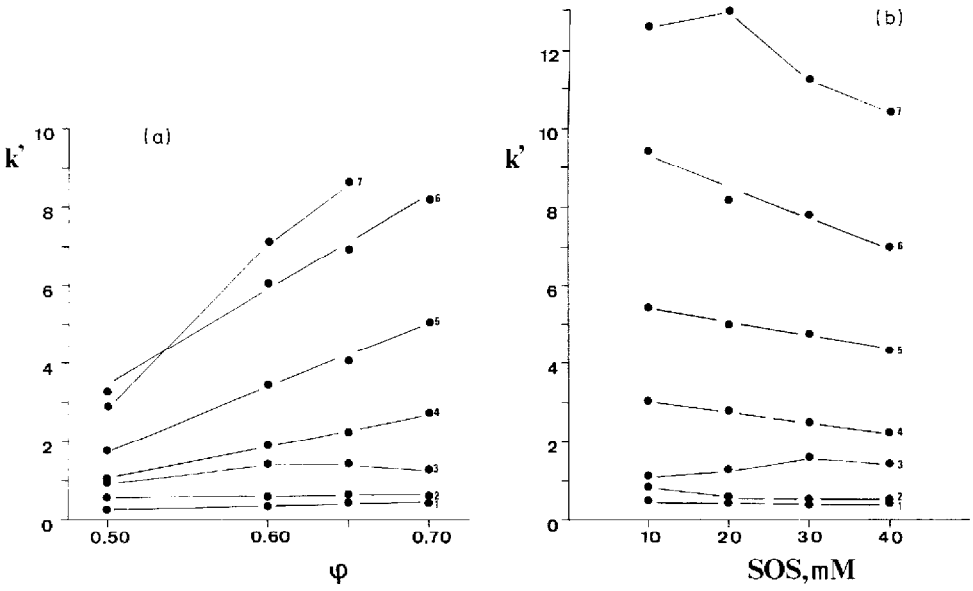

Fig. 1. Retention as a function of (a) the volume fraction of water (φ) and (b) the SOS concentration in the eluent. Mobile phase: (a) methanol–water containing 20 mmol/l of SOS and 5 mmol/l of DMOA (pH 3.0); (b) methanol–water (30:70) containing 5 mmol/l DMOA (pH 3.0). Column RSil C_{18} (5 μm) (150 × 4.1 mm I.D.); column temperature, 25°C; flow-rate, 1 ml/min; detection, 220 nm. Peaks: 1 = paracetamol; 2 = caffeine; 3 = codeine phosphate; 4 = acetylsalicylic acid; 5 = bromural; 6 = carbromal; 7 = salicylic acid; 8 = phenobarbital.

product of ASA, does not interfere with any of the active ingredients at sufficiently high water concentrations and under the conditions indicated in Fig. 1a, with φ = 0.60, all compounds could be baseline resolved except CAF–PAR. As the selectivity for this peak pair decreases with increasing φ whereas ASA and COD coelute and SAL interferes with the elution of CAR at lower φ values, other eluent parameters were varied.

As expected, increasing the apparent pH of the eluent decreased the retention of the acids SAL and ASA. The retention of all other compounds was independent of pH changes, except COD, which showed an increased retention at pH 6. However, the selectivity changes that resulted from increases in pH were such that the separation of the seven compounds of interest became even more critical compared with that obtained at pH 3.0. Hence, further experiments were performed at pH 3.0.

The variation of retention with SOS concentration revealed two important selectivity changes (Fig. 1b): the resolution between ASA–COD and CAF–PAR in-

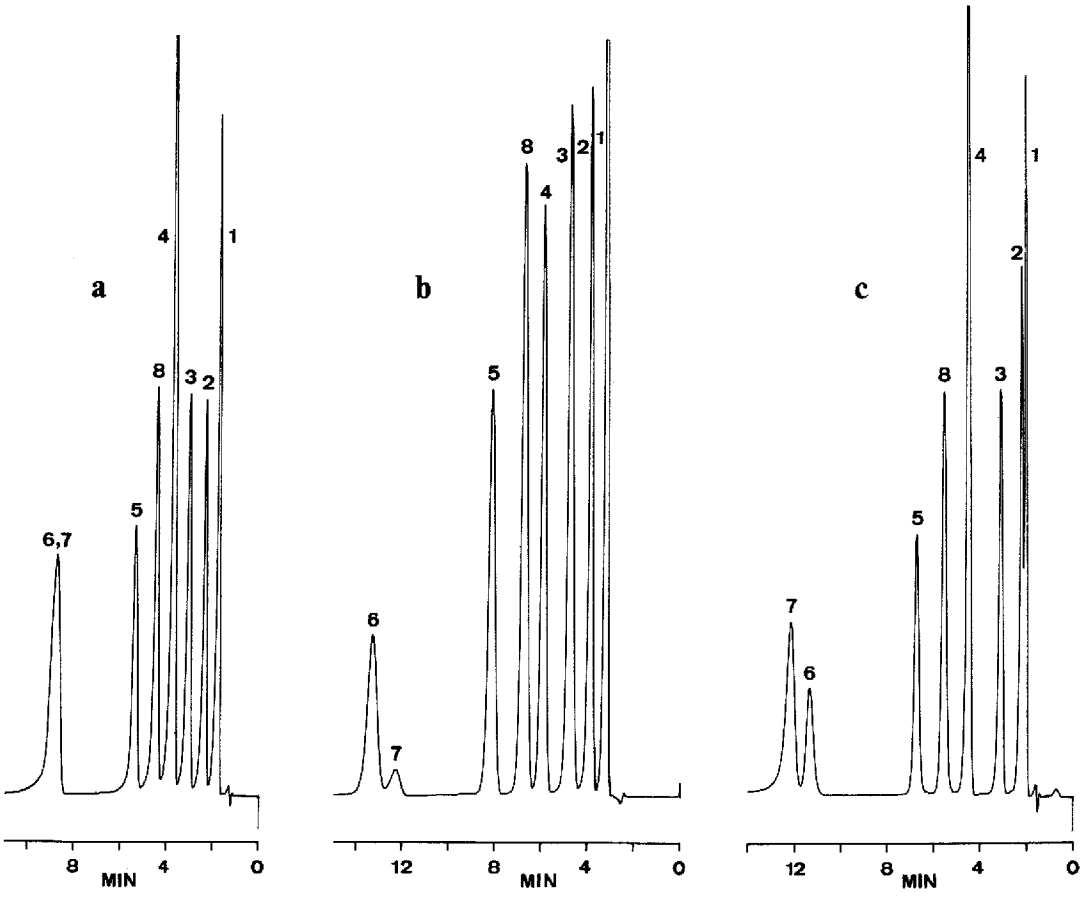

Fig. 2. Separation of a standard mixture of weak analgesic, hypnotic, sedative and stimulant drugs. Mobile phase: (a and c) methanol–water (40:60) containing 12.5 mmol/l of SOS and 5 mmol/l of DMOA (pH 3.0); (b) as (a) and (c), except φ = 0.50. Column: (a) as in Fig. 1; (b) RSil C_{18} (10 μm) (250 × 4.6 mm I.D.), (c) LiChrosorb RP-18 (5 μm) (150 × 4.6 mm I.D.). Chromatographic conditions and peaks as in Fig. 1.

creased with decreasing SOS concentration. In general, the retention decreased with increasing SOS content, except for COD, which was more strongly retained whereas the elution of PAR was not affected by the SOS content of the eluent.

The influence of DMOA concentration on retention was only briefly investigated as a concentration less than 2.5 mmol/l could not be used in order to avoid unacceptable peak tailing for COD and concentrations higher than 5 mmol/l reduced the resolution between CAF, COD and PAR. It should be stressed, however, that increasing the DMOA concentration can introduce important selectivity changes by increasing the retention of the acidic compounds and decreasing that of the basic compounds.

Attempts to use the described chromatographic conditions were succesful in resolving all active components of the mixture but under such conditions CAR coelutes with SAL (Fig. 2a). No better results could be obtained using various combinations and concentrations of mobile phase modifiers.

From previous results it could be deduced that the maximum overall selectivity could be expected at a relative low volume fraction of water, φ, and a low SOS concentration in the eluent. Such chromatographic conditions, however, could not be used on a short column (150 × 4.1 mm I.D.) as the retention became so low that PAR eluted near the dead volume of the column and insufficient separation was obtained for COD–CAF and ASA–COD. It was therefore decided to use a column with appropriate dimensions, containing the same stationary phase material, but with 10-μm instead of 5-μm particle diameter. Fig. 2b shows the resulting chromatogram of a separation of the analgesic mixture using an appropriate eluent composition. It is apparent that the baseline separation obtained and the sufficient separation of PAR from the dead volume of the reversed-phase column will permit the simultaneous determination of these drugs in pharmaceuticals.

Chromatography on other RP stationary phases

Commercially available reversed phases can differ considerably in their carbon load and degree of derivatization and the contribution to retention of residual polar groups may significant affect the resolving power of a chromatographic system[12,13]. To evaluate such effects, LiChrosorb RP material was selected, as it is a non-end-capped RP silica and increased polar interactions can be expected in comparison with RSil RP silica, which is end-capped. Elution was performed on LiChrosorb RP-18 (5 and 10 μm) and on LiChrosorb RP-8 (10 μm) stationary phases, using the chromatographic conditions in Fig. 2a. The elution sequence of the solutes investigated was the same on all of the supports tested. Chromatograms obtained on LiChrosorb RP-18 and RP-8 (both 10 μm) columns (250 × 4.6 mm I.D.) were very similar with respect to both the retention capacity and selectivity: SAL was not resolved from CAR in either instance and CAF–PAR were incompletely separated on RP-8, unlike on RP-18. Decreasing φ to 0.5 as in Fig. 2b results in insufficient resolution of the early eluting compounds in both instances.

The separation obtained on LiChrosorb RP-18 (5 μm) is shown in Fig. 2c to facilitate comparison with that obtained on RSil (Fig. 2a). The overall increase in retention observed on LiChrosorb might be due to the different column packing material, showing a higher retention for the compounds studied. CAR and SAL were sufficiently resolved but CAF and PAR almost coeluted. These results suggest that

polar activities do not significantly affect the separation selectivity or at least the combined effects of DMOA and SOS can adequately eliminate such interactions, as (weakly) basic compounds are extremely sensitive to such interactions, mostly resulting in long elution times and tailing peaks. We conclude that the separation can be repeated on LiChrosorb RP-18 (5 μm) using appropriate eluent compositions. No attempt was made, however, to optimize further the separation presented in Fig. 2c.

Applications

The liquid chromatographic behaviour of several other drugs frequently administered simultaneously with one (or several) of the drugs mentioned in Fig. 2. was studied. It is not possible to give exact chromatographic data about the separation of all these drugs, as the chromatographic conditions for a particular compound are partly determined by the accompanying drugs in the pharmaceutical preparation under investigation. However, it can be stated that any mixture containing a realistic

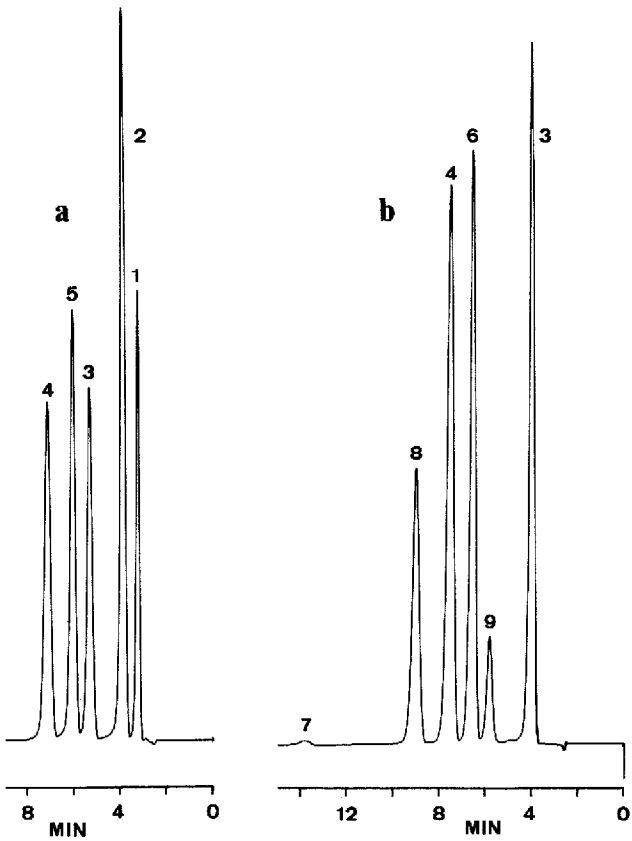

Fig. 3. Separation of all active drugs in (a) Gentarol® and (b) Salydent®. Mobile phase: (a) methanol–water (50:50) containing 15 mmol/l of SOS and 5 mmol/l of DMOA (pH 3.0); (b) as in (a) but containing 5 mmol/l of SOS and 5 mmol/l of DMOA. Column and chromatographic conditions as in Fig. 2b. Peaks: 1 = PAR; 2 = CAF; 3 = COD; 4 = PHO; 5 = SAA; 6 = ASA; 7 = SAL; 8 = PHE; 9 = HYO.

number of the drugs discussed above and allobarbital, butalbital, phenazone, aminophenazone, phenacetin, hyoscyamine, salicylamide and quinine can be separated such that simultaneous determination becomes possible. This statement includes about twenty commercially available pharmaceuticals, *e.g.*, Perdolan®, Veganine®, Codis®, Dolviran® and Neuridon®. An illustration of the separation possibilities with the proposed approach for two commercially available pharmaceuticals is given in Fig. 3.

Special attention was also paid to the analysis of pharmaceuticals containing quaternary ammonium drugs (QADs) and weakly basic analgesics. The separation of such multi-component pharmaceuticals requires a sufficiently high water content ($\varphi > 0.5$) to avoid coelution and/or elution in the dead volume of the early eluting drugs, and a low SOS content (2.5 mmol/l) and a relatively high DMOA concentration (5 mmol/l) to ensure fast elution of the QADs. As under these conditions COD and CAF are not completely resolved at pH 3.0, the pH was adjusted to 5.7 to provide

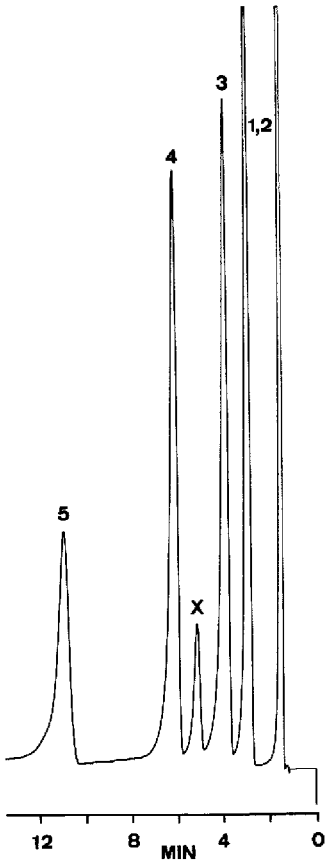

Fig. 4. Separation of a standard mixture of quaternary ammonium and weak analgesic drugs. Mobile phase: methanol–water (35:65), containing 2.5 mmol/l of SOS and 5 mmol/l of DMOA (pH 5.7). Chromatographic conditions and column as in Fig. 1. Peaks: 1 = dipyrone; 2 = caffeine; 3 = codeine phosphate; 4 = aminopyrine; 5 = tiemonium iodide; X = unidentified impurity of DIP. CAF, COD, AMI, TIE and DIP, COD, TIE are the active ingredients of Regisan® and Visceralgine® Compositum, respectively.

adequate selectivity. The resulting separation will allow the simultaneous determination of all components in the pharmaceuticals studied (Fig. 4). The coelution of DIP and CAF was not of practical importance because to our knowledge no pharmaceuticals containing DIP, CAF and QADs are marketed. All drugs shown in Fig. 4 can be eluted as pure compounds using 2.5 mmol/l DMOA in the eluent but the analysis time then increases to about 20 min.

CONCLUSIONS

The simultaneous addition of SOS and DMOA to RP liquid chromatographic systems can provide adequate resolution of mixtures containing basic, acidic and neutral drugs. About twenty commercially available pharmaceuticals, each containing several weak analgesics and related drugs, including hypnotics, sedatives, spasmolytics and barbiturates, were successfully analysed by this method.

REFERENCES

1 M. G. Mamolo, L. Vio and V. Maurich, *Farmaco, Ed. Prat.*, 40 (1985) 111.
2 M. G. Mamolo, L. Vio and V. Maurich, *J. Pharm. Biomed. Anal.*, 3 (1985) 157.
3 W. E. Wallo and A. D'Adamo, *J. Pharm. Sci.*, 71 (1982) 1115.
4 R. Thomis, E. Roets and J. Hoogmartens, *J. Pharm. Sci.*, 73 (1984) 1830.
5 T. D. Wilson, *J. Liq. Chromatogr.*, 9 (1986) 2309.
6 V. Das Gupta, *J. Pharm. Sci.*, 69 (1980) 110.
7 P. Cockaerts, E. Roets and J. Hoogmartens, *J. Pharm. Biomed. Anal.*, 4 (1986) 367.
8 J. A. De Schutter and P. De Moerloose, *Chromatographia*, 23 (1987) 667.
9 J. A. De Schutter and P. De Moerloose, *J. Chromatogr.*, 437 (1988) 83.
10 A. Albert and E. P. Serjeant, *The Determination of Ionization Constants*, Chapman and Hall, New York, 3rd ed., 1984, p. 166.
11 *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, Lippincott, Philadelphia, Toronto, 8th ed., 1982, p. 841.
12 W. A. Moats and L. Leskinen, *J. Chromatogr.*, 386 (1987) 79.
13 J. Köhler and J. J. Kirkland, *J. Chromatogr.*, 385 (1987) 125.