Journal of Pharmaceutical & Biomedical Analysis
Vol. 3, No. 5, pp. 439–445, 1985
Printed in Great Britain

0731–7085/85 $3.00 + 0.00
Pergamon Press Ltd.

Simultaneous determination of codeine and ibuprofen in plasma by high-performance liquid chromatography

RON GINMAN*[1], H. THOMAS KARNES[2] and JOHN PERRIN[2]

[1] *Department of Pharmacy, Brighton Polytechnic, Moulsecoomb, Brighton BN2 4GJ, UK*
[2] *College of Pharmacy, Box J-4, J. Hillis Miller Health Center, University of Florida, Gainesville, FL 32601, USA*

Abstract: A procedure is described for the simultaneous determination of codeine and ibuprofen in human plasma following the administration of the two substances in a proposed combination dosage form. The two substances were extracted separately from plasma and then determined together by high-performance liquid chromatography (HPLC) using a fluorescence detector. The codeine was first extracted from alkalinized plasma with hexane–dichloromethane (2:1, v/v) and then washed with sodium hydroxide solution. The ibuprofen was then extracted with hexane from the plasma acidified with sulphuric acid. The organic layers were collected, evaporated to dryness and the reconstituted residue was subjected to HPLC. The detection limit for codeine was 8 μg l^{-1} and for ibuprofen 1 mg l^{-1}.

Keywords: *Codeine; ibuprofen; reversed-phase high-performance liquid chromatography.*

Introduction

The combination of codeine (a narcotic analgesic with anti-tussive properties) and ibuprofen (a non-steroidal anti-inflammatory drug with analgesic and antipyretic activity) into a single dosage form is commercially contemplated; in consequence a method that is specific and quantitative for the measurement of the two drugs in blood will be required. Since the proposed drug combination is new no analytical method is available.

Codeine has been determined in plasma by reversed-phase high-performance liquid chromatography after extraction of the base [1, 2]. Tsina [1] discusses some of the advantages of this approach over others such as thin-layer chromatography, gas chromatography and gas chromatography–mass spectrometry.

The more recently available drug, ibuprofen, has been assayed by extracting the drug from acidified plasma with an organic phase before reversed-phase high-performance liquid chromatography [3–7]. Other approaches were to analyse the serum directly [8] or to absorb ibuprofen on to a resin prior to chromatography [9].

*To whom correspondence should be addressed.

Attempts were made to determine codeine and ibuprofen simultaneously in plasma by forming an ion-pair complex of codeine with sodium 1-octane sulphonate and extracting this complex together with the ibuprofen from acidified sera. The two extracted drugs were analysed by reversed-phase liquid chromatography. This approach proved to be less than satisfactory because it afforded poor drug recovery from spiked plasma. However, the method described fully in the present work, where codeine and ibuprofen are extracted separately with appropriate adjustment of the pH of the sera, proved satisfactory in terms of good recovery as well as precision. In the method the individual extracts were concentrated, combined and then analysed for the drugs by reversed-phase liquid chromatography with fluorimetric detection.

Materials and Method

Chemicals

Codeine phosphate and flurbiprofen were supplied by Boots Company (USA) and ibuprofen by Upjohn Company; *N*-isopropylcodeine was prepared by K. B. Sloan. All reagents and solvents were of reagent grade. All solutions were prepared with de-ionized distilled water unless otherwise stated. Acetonitrile was HPLC grade and all aqueous solutions for HPLC were filtered through a 0.45-μm nylon 66 membrane (Rainin Instruments). The mobile phase was acetonitrile–0.1 M potassium dihydrogen phosphate with 0.05 M sodium octane sulphonate (pH adjusted to 3.7 with concentrated phosphoric acid) (22:78, v/v).

Apparatus

The apparatus was a Varian high-performance liquid chromatograph, model 5000, fitted with a 20-μl sample loop and with a proportioning pump for delivering a set composition of acetonitrile to aqueous phase. The detector, a Schoeffel FS 970 spectrofluorimeter, was set for excitation at 213 nm with no emission filter. For the concentrations of the drugs used the sensitivity settings of the detector were 0.02, 0.025 and 0.1 μA full-scale deflection. The 300 × 4 mm i.d. column was packed with 10-μm reversed-phase Varian Micropak (CN10). All tubes used in the extractions had well-fitting screw caps.

Chromatographic procedures

The sample of plasma (2 ml) was spiked with 20 μl of the aqueous solutions of the internal standards, *N*-isopropylcodeine (30 mg l^{-1}) and flurbiprofen (16 mg l^{-1}). The mixture was made alkaline with 2 ml of 50 mM phosphate buffer (pH 8). Codeine was extracted with two 6-ml portions of hexane:dichloromethane (2:1, v/v). In each extraction the mixture was shaken by hand for 2 min, centrifuged and the organic layer removed. The organic layer (12 ml) was shaken with 1 ml of 0.05 M sodium hydroxide for 2 min and again separated. The organic layer in a 12-ml tube was then evaporated to dryness under a gentle stream of nitrogen using a water-bath at 30°C.

The alkaline plasma was acidified with 0.25 ml of 2 M sulphuric acid and 12 ml of hexane was added. The two phases were equilibrated by gentle mixing for 1 h on a rotator; 8 ml of the organic layer was removed and added to the dried codeine extract. Gentle evaporation under dry nitrogen was carried out until 1 ml of solution remained. The sides of the tube were washed down with about 1.5 ml of hexane before the contents were evaporated to dryness.

The residue was dissolved in 100 μl of acetonitrile–water (1:1, v/v) and 20 μl of this solution was subjected to chromatography. The column was at room temperature and the flow rate of the mobile phase was 1.8 ml min^{-1}.

For the establishment of standard curves, the above procedure was carried out using drug-free plasma spiked with codeine and ibuprofen to yield a final drug concentration in the plasma of 10–100 μg l^{-1} and 10–80 mg l^{-1}, respectively. Peak-height ratios were obtained by dividing the peak height of codeine by that of *N*-isopropylcodeine and the peak height of ibuprofen by that of flurbiprofen.

Results and Discussion

Early attempts to evaluate codeine and ibuprofen simultaneously by the same chromatographic procedure proved successful. The method consisted of the precipitation of protein in the plasma by the addition of 2 ml of acetonitrile to 1 ml of plasma, mixing with a vortex mixer for 5 min and then centrifuging. The supernatant liquid was transferred to another tube and 1 ml of hexane was added. After mixing in a vortex mixer for 15 s, the two phases were allowed to separate. The hexane layer was removed and discarded. The lower layer was evaporated to dryness. The dried residue was reconstituted immediately before HPLC analysis with acetonitrile–distilled water (35:65, v/v). The mobile phase was the same as that described under "Chemicals" except that the pH was not adjusted to 3.7 with concentrated phosphoric acid but left at the phosphate pH of 4.6. Both UV (Varichrome) and fluorescent detectors (Schoeffel) were used. A typical chromatogram is shown in Fig. 1 where flurbiprofen was used as the internal standard; it will be noted that good separation was obtained between codeine and norcodeine (a metabolite of codeine). It was also found that codeine could be readily determined just as well by fluorescence as by UV. However, because of their significant solubilities in hexane, only 40% recovery of the two drugs was achieved. This loss to the hexane led to poor sensitivity in the determination of codeine and ibuprofen in plasma. If the washing with hexane was omitted, it was found that the non-polar plasma constituents interfered with the chromatographic peaks of codeine and flurbiprofen.

Other methods of protein precipitation [10] were tried without success, for example, the addition of perchloric acid led to degradation of codeine and concentrated hydrochloric acid resulted in a poor recovery. Other organic solvents (e.g. chloroform and dichloromethane) used for extraction gave "stable" emulsions on mixing or shaking. It was decided therefore that the best approach was first to extract codeine from alkalinized plasma, then to extract iboprofen from acidified plasma and finally to combine the extracts before chromatography.

In the extraction of codeine from plasma it was found that too violent or prolonged shaking or mixing by a vortex mixer caused the formation of a stiff gel throughout the organic layer. The gel could be somewhat broken by mechanical shock to the tube. The 2-min hand shaking recommended by Aarons *et al.* [4] minimized the chances of gel formation. For extraction of the acid, shaking should be gentle and prolonged to give complete transfer and little formation of a gel in the hexane; 1 h on a rotator was found to give the optimum time for good recovery. A typical chromatogram is shown in Fig. 2. Codeine has a pKa of 8.2 and would therefore be fully ionized at the pH of the mobile phase and would readily form an ion-pair with sodium octane sulphonate. The formation of the ion-pair enabled codeine to be analysed together with ibuprofen.

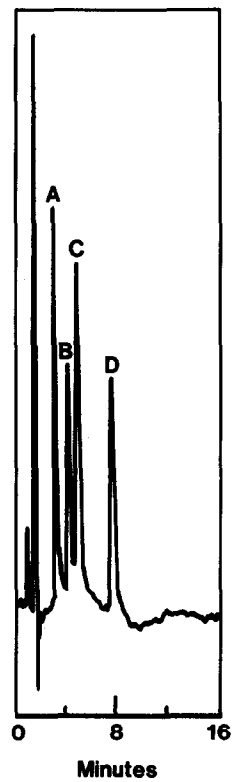

Figure 1
Chromatogram of (A) norcodeine (20 μg ml^{-1}), (B) codeine (2 μg ml^{-1}), (C) internal standard flurbiprofen (2.5 μg ml^{-1}) and (D) ibuprofen (2 μg ml^{-1}) in acetonitrile:water (1:1, v/v). The column was a Varian Micropak CN10, 300 × 4 mm i.d. Detection was by Varichrome UV at 220 nm and a sensitivity of 0.01. The mobile phase was acetontrile–0.01 M potassium hydrogen phosphate with 0.05 M sodium 1-octane sulphonate (pH 4.6) (32:68, v/v).

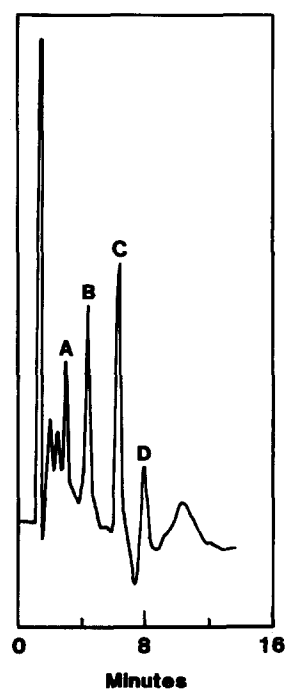

Figure 2
Chromatogram of (A) codeine (60 μg l^{-1}), (B) *N*-isopropylcodeine (300 μg l^{-1}), (C) flurbiprofen (160 μg l^{-1}) and (D) ibuprofen (16 mg l^{-1}), extracted from plasma (2 ml). The chromatographic conditions were the same as given in Fig. 1 except that 22% v/v of acetonitrile was used and the aqueous phase was adjusted to pH 3.7.

With the chosen composition of the mobile phase (22% v/v of acetonitrile; pH of the aqueous phase 3.7) the retention time of codeine was sufficiently long (about 4 min) to keep its peak clear of those of the plasma constituents while the retention times of ibuprofen (about 8 min) and the internal standard (about 6 min) were not excessively long. It was found that the retention time of flurbiprofen altered more significantly with changes in mobile phase composition than did that of ibuprofen, so much so, that at concentrations of less than 20% v/v of acetonitrile, flurbiprofen had relatively longer retention times than did ibuprofen.

Standard curves established by spiking drug-free plasma with codeine and ibuprofen as well as the internal standards were linear over the concentration ranges expected in plasma, 0–100 $\mu g\ l^{-1}$ for codeine and 0–80 $mg\ l^{-1}$ for ibuprofen. The correlation coefficients of the calibration curves were 0.9998 and 0.9999 for codeine and ibuprofen, respectively. The precision of the method was assessed using the data for plasma spiked with the two drugs. Tables 1 and 2 show the between-assay relative standard deviation

Table 1
Precision* for the determination of codeine in drug-free plasma spiked with codeine

Codeine concentration in plasma (μg/l)	Mean peak-height ratio†	RSD‡ (%)
10	0.106	4.7
25	0.192	4.4
50	0.321	2.3
80	0.507	3.6
100	0.648	1.6

*The regression equation for peak-height ratio against concentration of codeine was: $y = 0.039 + 0.00596x$; the standard error (SE) of the gradient was ±0.00011 and the SE of the intercept was 0.07.
†Mean value of five determinations.
‡Relative standard deviation (%); $n = 5$.

Table 2
Precision for the determination of ibuprofen in drug-free plasma spiked with ibuprofen

Ibuprofen concentration in plasma (mg/l)	Mean peak-height ratio†	RSD‡ (%)
10	0.915	4.0
20	1.48	1.4
40	2.59	2.1
60	3.75	2.8
80	4.93	1.3

*The regression equation for peak-height ratio against concentration of ibuprofen was: $y = 0.328 + 0.0573x$; the SE of the gradient was ±0.0005 and the SE of the intercept was 0.0252.
†Mean value of five determinations.
‡Relative standard deviation (%); $n = 5$.

(RSD) at each concentration. With a signal-to-noise ratio of 2, the lower limits of quantitation were estimated to be 8 $\mu g\ l^{-1}$ for codeine and 1 $mg\ l^{-1}$ for ibuprofen. There was no loss of codeine or ibuprofen in plasma when stored in a deep freeze for 3 weeks. The total recovery of codeine and ibuprofen for the whole extraction and evaporation to the final solution for injection into the chromatograph was 85 ± 1% and 89 ± 2%, respectively (n = 5 for the five concentrations measured for each drug). Analysis time and the amount of acetonitrile consumed can be significantly reduced if the dried extracts of the two drugs are combined before chromatography as described under Chromatographic procedures.

The metabolite, norcodeine, could appear in the plasma samples, and if so would be at a very low concentration [11]. However, in order to determine if norcodeine contributed to the codeine peak, samples of codeine solution were spiked with norcodeine. The chromatogram showed good resolution between the two narcotics (Fig. 3).

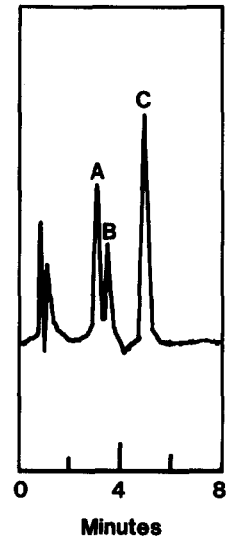

Figure 3
Separation of (A) norcodeine and (B) codeine under the conditions given in Fig. 2. The internal standard, *N*-isopropylcodeine, is depicted by (C).

Some plasma samples showed a negative peak between the peaks of ibuprofen and flurbiprofen. This phenomenon was found to be due to the presence of caffeine in the plasma [1] and could be overcome by: changing the composition of the mobile phase; carrying out chromatography of the ibuprofen and flurbiprofen separately from codeine and its co-extractants; and restraining the subject from imbibing caffeinated drinks well before blood samples are taken. Since chromatography resolved ibuprofen and flurbiprofen, the method can be used to determine flurbiprofen with ibuprofen as the internal standard. Under such conditions there would be no need to use sodium octane sulphonate.

Acknowledgements: The authors express their thanks to K. B. Sloan of the College of Pharmacy, Florida for preparation of *N*-isopropylcodeine and to C. M. Riley for helpful advice. Also we wish to thank Boots Company (USA) for financial support.

References

[1] I. W. Tsina, M. Fass, J. A. Debban and S. B. Matin, *Clin. Chem.* **28**, 1137–1139 (1982).
[2] J. Visser, G. Grasmeijer and F. Moolensaar, *J. Chromatogr.* **274**, 372–375 (1983).

[3] J. A. Stead, M. Freeman, E. G. John, G. T. Ward and B. Whiting, *Int. J. Pharm.* **14**, 59–72 (1983).
[4] L. Aarons, D. M. Grennan, C. Rajapakse, J. Brinkley, M. Siddiqui, L. Taylor and C. Higham, *Brit. J. clin. Pharmacol.* **15**, 387–388 (1983).
[5] G. F. Lockwood and J. G. Wagner, *J. Chromatogr. (Biomed. Appl.)* **232**, 335–343 (1982).
[6] G. L. Kearns and J. T. Wilson, *J. Chromatogr. (Biomed. Appl.)* **226**, 183–190 (1981).
[7] J. L. Shimek, N. G. S. Rao and S. K. Wahba Khalil, *J. Pharm. Sci.* **70**, 514–516 (1981).
[8] A. Ali, S. Kazmi and F. M. Plakogiannis, *J. Pharm. Sci.* **70**, 944–945 (1981).
[9] B. G. Snider, L. J. Beaubien, D. J. Sears and P. D. Rahn, *J. Pharm. Sci.* **70**, 1347–1349 (1981).
[10] J. Blanchard, *J. Chromatogr. (Biomed. Appl.)* **226**, 455–460 (1981).
[11] M. K. Brunson and J. F. Nash, *Clin. Chem.* **21**, 1956–1960 (1975).

[*First received for review 10 July 1984; revised manuscript received 22 January 1985; final version received 7 March 1985*]