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Short Communication

High performance liquid chromatographic analysis of a multicomponent product using a silica stationary phase and an aqueous–organic mobile phase

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Introduction

Elution behaviour of a drug substance in liquid chromatography (LC) depends on the interaction between the substance, the mobile phase and the stationary phase. Because of these interactions, isocratic LC assays of multicomponent drug products often require a compromise among the optimal chromatographic conditions for each drug. The analgesic combination in this report contains acetaminophen, butalbital and caffeine (Fig. 1), three drugs of widely different physicochemical properties. Butalbital is a weak acid of pK_a 7.6 [1, 2]. In contrast, acetaminophen is neutral and caffeine is slightly basic. Also, butalbital is only slightly soluble in water (1:>100), compared with acetaminophen (1:70) and caffeine (1:50) [2]. Because of these differences, simultaneous LC of the three compounds is difficult. For instance, attempts in this laboratory to adapt Rosenbaum's method [3] for aspirin, caffeine, butalbital and phenacetin to the analgesic combination in this study were unsuccessful due to close elution of acetaminophen and caffeine. Upon further modification of the method, both compounds were resolved, but excessive retention (>15 min) and tailing of the butalbital peak resulted. Other published approaches for analysing combinations containing butalbital include use of an alkaline mobile phase [3] and gradient LC [4]. Both these alternates involved additional column, instrument problems or both [5, 6]. The approach presented in this report is based on manipulation of the drug–mobile phase–stationary phase interactions by use of a silica column in the 'reversed-phase mode'. Such use

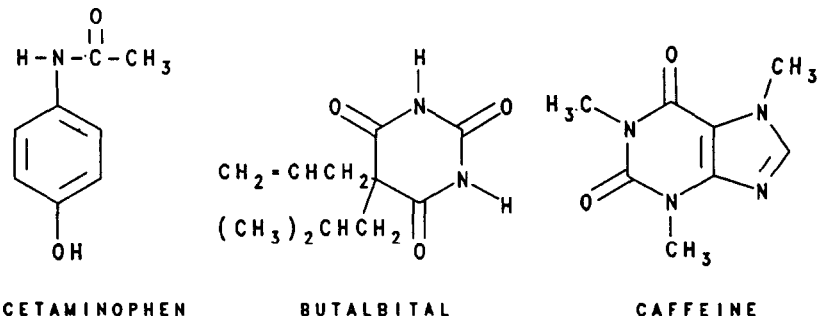

Figure 1
Chemical structures of acetaminophen, butalbital and caffeine.

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of silica columns for LC of basic amino compounds is well documented [7–12].

Experimental

Reagents and chemicals

Deionized water (Milli-Q Reagent Water System, Millipore Corp.), 85% phosphoric acid, methanol and isopropanol (HPLC grade, Fisher Chemical Co.). Acetaminophen (AP), butalbital (BU) and caffeine (CA) standard powders were USP grade. All materials were used as received. The tablet formulations were prepared using FDA approved excipients.

Apparatus

The LC system consisted of a Model 114M liquid chromatograph pump (Beckman Instruments) Model LC 241P autoinjector (Dynatech Precision Sampling) fitted with a 10-μl injection loop, Model 737 absorbance detector (Applied Biosystems, Ramsey Analytical Division) equipped with a 12-μl flow cell and operated at a wavelength of 214 nm, sensitivity of 0.5 AUFS and a filter rise time of 1.0 s, and a Model SP4270 Data Integrator (Spectra-Physics) at a chart speed of 2.5 mm min^{-1}. A Beckman Model ϕ71 pH meter was used to determine the pH of the aqueous solutions. The column used was a stainless steel column, 25 cm length × 4.6 mm i.d. containing 5 μm Zorbax SiL spherical silica (DuPont). The mobile phase consisted of dilute aqueous phosphoric acid (pH 2.1)–methanol–isopropanol (95:3:2, v/v/v). Dilute aqueous phosphoric acid was prepared by adding sufficient 85% phosphoric acid to HPLC grade water to bring the pH to 2.1. The flow rate was 1.5 ml min^{-1} under ambient temperature conditions.

Sample preparation

All stock solutions and dilutions were prepared in deionized water and filtered through Acrodisc (Gelman) PTFE membrane filters (0.45 μm). Tablet samples were finely ground before analysis and stirred magnetically for at least 1 h to ensure complete solution.

Method development and validation

The effect of mobile phase composition on capacity factor (k') and resolution (R) among the drug peaks was investigated. Experiments were also conducted to determine the linearity, precision, sensitivity and accuracy of the assay method. These studies followed previously published guidelines for method validation [13].

Results and Discussion

Method development

Silica columns from three manufacturers — DuPont (Zorbax), Merck (Lichrosorb) and Beckman (Altex) were initially investigated. The latter two columns were found to be unsuitable due to difficulty in separation of acetaminophen and butalbital. Excellent baseline separation of the three drugs was obtained on the Zorbax column with a mobile phase consisting of dilute aqueous phosphoric acid, isopropanol and methanol in the ratio 95:3:2 (Fig. 2). As shown in Table 1 and Figs 3 and 4, higher capacity factors (k') for all three compounds were obtained with 5% methanol, but the resolution between butalbital and caffeine was reduced. With 5% isopropanol, resolution between acetaminophen and butalbital decreased significantly. The observed elution order (acetaminophen > butalbital > caffeine) presumably reflects the combined influence of the aqueous solubilities of the three drugs and their dissociation constants. At the acid pH of the mobile phase, acetaminophen and butalbital are unionized and are therefore retained in inverse order of their aqueous solubilities. Caffeine, a weak base, is retained to a greater extent than either of the other two drugs despite its ionization in the acidic mobile phase.

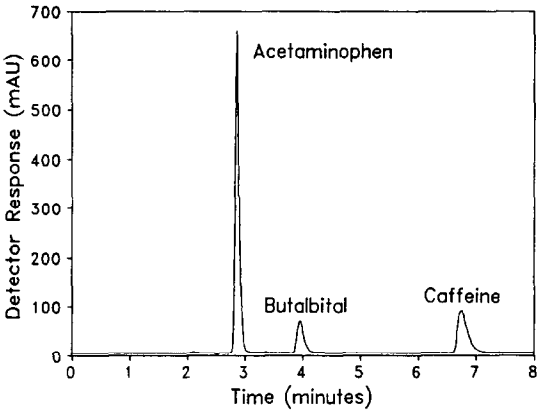

Figure 2
Chromatogram of an aqueous solution of acetaminophen, butalbital and caffeine on a Zorbax SiL column, (5 μm, 250 × 4.6 mm i.d.) using phosphoric acid (pH 2.1)–methanol–isopropanol (95:3:2) as mobile phase at a flow rate of 1.5 ml min^{-1}, ambient temperature and a detector wavelength of 214 nm.

Table 1
Effect of methanol–isopropanol ratio on chromatographic parameters

Methanol–isopropanol ratio	k'			R_s*	
	AP	BU	CA	AP–BU	BU–CA
5:0	0.35	2.28	2.71	8.52	1.41
0:5	0.21	0.50	0.98	2.10	3.38
3:2	0.24	0.77	1.38	3.41	4.71

AP = acetaminophen, BU = butalbital and CA = caffeine.
* $R_s = 2\left[\frac{tr_2 - tr_1}{W_1 + W_2}\right]$.

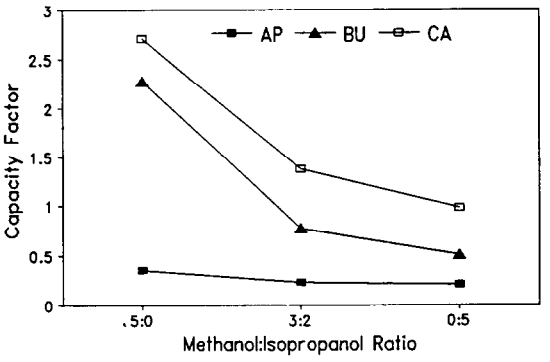

Figure 3
Effect of methanol–isopropanol ratio on capacity factor for acetaminophen (AP), butalbital (BU) and caffeine (CA).

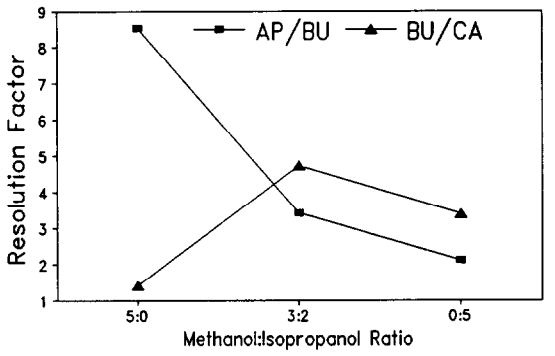

Figure 4
Effect of methanol–isopropanol ratio on resolution factor for acetaminophen (AP), butalbital (BU) and caffeine (CA).

The retention of caffeine was explained in terms of electrostatic (ion-exchange) interactions with the anionic silanol groups [8].

Method validation

Several articles have been published on analytical-method validation and most include tests for linearity, accuracy, precision, reproducibility, sensitivity and specificity [14–19]. For this method, the concept of tailoring a validation scheme to the intended use of the method, discussed by Williams [18], was adopted.

Linearity. The correlation coefficient values ($r \geq 0.999$) and the good agreement between the actual and calculated peak areas indicate that all three drugs have linear concentration versus peak area relationships, in the relevant concentration ranges investigated (Table 2 and Fig. 5). The plots indicate constancy of detector response for butalbital and caffeine over the entire concentration range, but a slight negative deviation from linearity at the two highest concentrations of acetaminophen. The concentration ranges represent 20–150% of the concentration of a 1 l solution of a tablet containing 325 mg acetaminophen, 50 mg butalbital and 40 mg caffeine. These ranges encompass the usual assay and tablet dissolution concentrations.

Precision. As an index of system precision, the RSD of five injections were calculated for the linearity samples. The RSD values ranged from 0.2 to 1.1% and were substantially lower than the recommended 2% system precision limit in the USP [20]. The method precision was determined by conducting replicate assays ($n = 10$) of a composite mix of crushed tablets from a single batch. This experiment presumed homogeneity of the tablet powder. The results (Table 3) indicated excellent reproducibility of the method with mean assay results of 97.6, 97.3 and 97.8% for acetaminophen, butalbital and caffeine, respectively. The RSD for each drug assay was <1%.

Sensitivity and accuracy. Method sensitivity was determined by a standard addition method. Sample solutions of known concentration were spiked with a standard solution of

Table 2
Linear regression of peak area versus concentration data

Drug	Correlation coefficient	Slope	Intercept
Acetaminophen*	0.99988	323.49	2437.1
Butalbital	0.99987	714.55	−23.5
Caffeine	0.99973	433.11	7.7

* Regression of first four data points only.

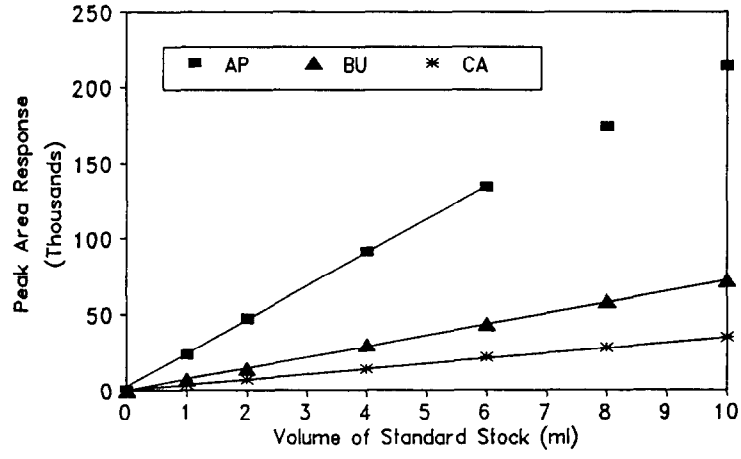

Figure 5
Plots of peak area responses versus volume of stock standard solution of acetaminophen (AP), butalbital (BU) and caffeine (CA). Solid lines are calculated from least-squares regression equations. Symbols represent mean values of peak areas (n = 5). Curves span 20–150% of the expected concentrations of the three components.

Table 3
Drug recovered from replicate assays of a single batch of crushed tablets (n = 10)

	Recovery (%)*		
Parameter	Acetaminophen	Butalbital	Caffeine
Mean	97.6	97.3	97.8
Max	99.0	98.5	99.0
Min	96.8	96.0	96.4
RSD	0.8	0.9	0.8

* Results expressed as per cent of labelled amounts of drug, i.e. acetaminophen: 325 mg; butalbital: 50 mg; caffeine: 40 mg.

Table 4
Drug recovered from sample solutions spiked with a standard solution at various levels (n = 2)

	Recovery (%)		
Spike*	Acetaminophen	Butalbital	Caffeine
5	100.4	98.0	99.2
10.0	100.4	101.1	99.8
15.0	100.4	101.1	101.9
Mean	100.4	100.0	100.0

* Added drug as per cent of original drug concentration in solution.

the three drugs at approximately 5, 10 and 15% of the sample concentrations. Assays of the spiked solutions resulted in recovery data between 98 and 101% of the expected amounts (Table 4). Thus, the method is sensitive to small percentage changes in drug concentration. Such sensitivity is especially important for butalbital, which, being a Schedule III controlled substance, is assayed at a specified percentage range narrower than the usual 95–105%. The data also indicated the accuracy of the method, since the amounts of spiked drug recovered corresponded to the added amounts.

Specificity. Studies to demonstrate the specificity of the assay in the presence of excipients and known degradants were carried out. In the excipient study, combinations of a mixture of tablet excipients and a solution of the drugs in various ratios were prepared and assayed for drug recovery. The excipient mix consisted of common tableting adjuvants such as a bulking agent, a disintegrant and a lubricant. The recovery data (Table 5) indicated that there was no interference by the excipients in assay of the drugs, even at four times the usual levels per tablet. Mean per cent

Table 5
Drug recovered from solutions containing various ratios of drugs and excipient (n = 2)

	Recovery (%)		
D–E ratio*	Acetaminophen	Butalbital	Caffeine
1:1	100.0	99.5	100.1
1:2	100.2	99.8	100.8
1:4	100.4	100.4	101.0
0.8:1.0	101.5	100.5	99.9
1.0:1.0	100.6	101.7	99.8
1.2:1.0	99.1	101.2	99.4

* Drug–excipient ratio based on the amounts per tablet.

drug recovered ranged from 99.5 to 101.7% for all three drugs. Under proper conditions of storage, butalbital and caffeine are chemically stable, even in solution at elevated temperatures [21, 22]. Acetaminophen, however, is susceptible to acid-catalysed hydrolysis, yielding *p*-aminophenol [23]. Our experience with aged (>6 months) solutions of each drug confirm this. Chromatograms of standard solutions spiked with *p*-aminophenol indicate baseline to baseline resolution of the degradant and acetaminophen. Also, chromatograms of forcibly degraded solutions (obtained by treatment with concentrated acid or base for 24 h) did not show other extraneous peaks (Fig. 6). The method is therefore specific for the three drugs under mild conditions of degradation.

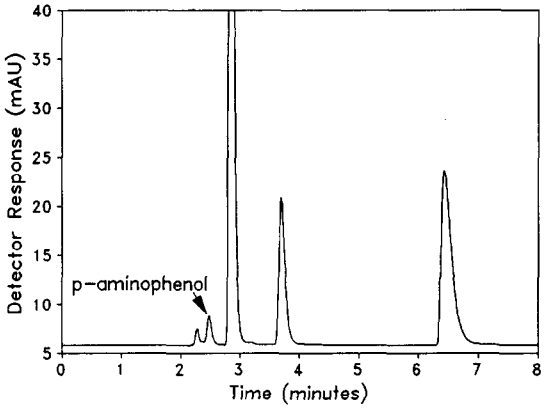

Figure 6
Expanded view of chromatogram of aged aqueous solution of acetaminophen, butalbital and caffeine treated with concentrated hydrochloric acid for 24 h. See Fig. 2 for conditions.

Conclusions

This LC method enabled rapid analysis of aqueous solutions of acetaminophen, butalbital and caffeine by use of a silica stationary phase and a mostly aqueous mobile phase. With this mobile phase, dissolution of the silica in water is a legitimate concern. The average pK_a of silica is 6.5 [24] and its solubility in water is significant at pH values >7.5. In this method, a pH was selected at which the silica is practically insoluble. This factor, combined with proper column care, enabled prolonged column life without significant degeneration in performance. In addition, this method did not require the typical long equilibration times required for silica columns. In fact, once the methylene chloride packing solvent was flushed from the column with isopropanol followed by isopropanol–water, equilibration typically required approximately 500 column volumes. The characteristic advantages of this method are its use of a readily available silica column, a simple, isocratic mobile phase and the short run time. This makes it especially useful in high throughput analytical laboratories.

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