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Mixed ion pair liquid chromatography method for the simultaneous assay of ascorbic acid, caffeine, chlorpheniramine maleate, dextromethorphan HBr monohydrate and paracetamol in Frenadol™ sachets

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Abstract: The five active drug substances and two of the excipients present in Frenadol™, a cold medication, were separated. The active drug components dextromethorphan HBr monohydrate, ascorbic acid, caffeine, paracetamol and chlorpheniramine maleate were quantitatively assayed by a mixed ion pair LC method. The excipients separated were citric acid and maleic acid. The HPLC assay included dual-wavelength detection to simultaneously quantify the large concentration of paracetamol and the much lower concentration of chlorpheniramine and dextromethorphan. Both tetrabutylammonium hydrogen sulphate (TBA) and pentane sulphonic acid (PSA) were necessary for resolution of the seven compounds. The TBA was necessary to lessen peak tailing for dextromethorphan and chlorpheniramine, to retain ascorbic acid and to shorten assay time. The pentane sulphonic acid enhanced peak shape for dextromethorphan and chlorpheniramine. The assay of the active drug substances was validated for use in quality control applications. Validation studies demonstrated that the procedure was accurate, linear, precise, reproducible and rugged. The method conformed to both USP and EC validation guidelines.

Keywords: *Ascorbic acid; chlorpheniramine maleate; caffeine; paracetamol; acetaminophen; dextromethorphan HBr; Frenadol™ sachets; quality control assay method.*

Introduction

Frenadol™ is an over-the-counter cold medication produced in Spain by Merck & Co., Inc. It contains the following active drug substances including ascorbic acid (ASC), chlorpheniramine maleate (CHL) as an antihistamine, caffeine (CAF) as a stimulant, paracetamol (PAR) or acetaminophen as an analgesic and dextromethorphan HBr monohydrate (DEX) as an antitussive. Three liquid chromatography (LC) methods, a colorimetric method and a titration method are currently used in quality control (QC) for the assay of the five active drug substances described. Clearly, greater efficiency could be achieved by using a single LC method for the assay of the five active ingredients. Methods exist in the literature for the assay of one or more of the Frenadol active components, but none claim to separate more than three of the five actives simultaneously without changing chromatographic conditions such as mobile phase or columns [1–4]. One of these methods [1] described a mobile phase and column which could be used to assay up to four of the Frenadol components individually, but not simultaneously. This method, in our hands, could not be used or modified for use as a simultaneous assay method for Frenadol. The analysis was further complicated by the presence of only 0.008 mg ml^{-1} of CHL versus 1.3 mg ml^{-1} of PAR which resulted in off-scale peaks of PAR when the concentration of CHL in samples was measurable. In order to address quality assurance requirements, a new ion pairing HPLC method, which assayed the five actives simultaneously in less than 15 min, has been developed and validated. Moreover, this method also separated the maleic acid and with minor modification could separate the citric acid which were used as excipients in the formulation.

Experimental

Equipment and materials

The LC method was developed using a Spectra Physics (San Jose, CA) gradient system consisting of a P4000 pump, an AS 3000 autoinjector (50 μl, complete loop filling) and

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a scanning Spectra Focus detector. Validation and ruggedness assays were performed using both the Spectra Physics instrument described above and a Waters (Milford, MA) HPLC system. The latter included a model 600 pump, model 712 WISP autoinjector (50 μl, partial loop filling) and a multiple wavelength model 490 detector. The columns utilized for the assays were end-capped, 5 μm particle size, Shandon Hypersil Phenyl-2 (4.6 mm × 25 cm). The columns were packed and supplied by Alltech (Deerfield, IL). Chemicals were production grade for spiked placebo validation assays except CAF which was USP grade. In ruggedness assays, all active drug substances were USP and excipients were production grade. The two ion pairing agents used were 97% pure tetrabutylammonium hydrogen sulphate (TBA) (Aldrich, Milwaukee, WI) and HPLC grade 1-pentane sulphonic acid (PSA, Sigma, St Louis, MO).

Mobile phase

The isocratic mobile phase consisted of 50 mM monosodium phosphate, 125 mM TBA and 1 mM PSA by weight in a solution of acetonitrile (ACN)–water (pH* 2.5) (5:95, v/v). The reagents were dissolved and the pH was adjusted with 1 N sodium hydroxide to the apparent pH* 2.5. The flow rate was maintained at 2 ml min^{-1} and the column temperature pre-set at 40°C.

Standards preparation

For placebo assays, standards were prepared at 50, 75, 100, 125 and 150% of the label claim. The label claim concentrations were as follows: DEX (0.04 mg ml^{-1}); ASC (0.5 mg ml^{-1}); PAR (1.3 mg ml^{-1}); CHL (0.008 mg ml^{-1}); CAF (0.03 mg ml^{-1}); and citric acid (0.03 mg ml^{-1}).

A single standard solution was used for composite and dose uniformity assays which contained all five actives at 100% of label claim (the sachet concentrations) and used citric acid as a buffer was prepared in water. Sachets were single dose unit, sealed foil pouches (10.5 × 5.5 × 0.5 cm).

Validation studies

Spiked placebos were prepared according to the manufacturing formula. For example, a placebo for PAR contained the excipients as well as the other four active drug substances present in the formulation at 100% of label claim. These spiked placebo solutions were tested at five levels: 50, 75, 100, 125 and 150% of label claim for each individual active. Assays were performed in duplicate on two samples at the five levels. This was repeated with a second instrument, mobile phase, standard and sample preparation, and analyst on different days. The complete set of validation assays was performed for each of the active drug substances in the presence of the 100% level of the other drug substances (100 injections of 50 samples).

Ruggedness testing

For composite assays, the contents of 10 sachets were combined, ground in a mortar and mixed before weighing a 10 g sample for assay. Two samples from each of three lots of Frenadol were assayed in duplicate injections by two analysts on two instruments using two separately prepared standard and mobile phase preparations (24 injections of 12 samples). For dose uniformity assays, individual Frenadol sachets (10 g each) with 20 mg of DEX, 250 mg of ASC, 15 mg of citric acid, 15 mg of CAF, 4 mg of CHL, 650 mg of PAR, 8.2 g of sucrose and 0.846 g of other excipients such as flavours were dissolved in 500 ml of water and sonicated for 15 min. This was the 100% label claim concentration (see above) with excipients. Although all the Frenadol components were soluble, samples were passed through 0.45 μm filters before HPLC assay. Ten Frenadol sachets from each batch were assayed in duplicate injections by two analysts (120 injections of 60 samples).

Results and Discussion

Methods development

Initial separations were conducted with a μBondapak phenyl column (Waters, Milford, MA), 5 mM 1-pentane sulphonic acid ion pairing agent in acetonitrile–water (25:75, v/v) with sodium phosphate buffer pH* 2.2 at monosodium phosphate concentrations from 10 to 100 mM. Under these conditions only DEX and CHL were retained. It was determined that 50 mM phosphate yielded optimal peak shape and enhanced stationary phase binding by the uncharged molecule PAR, but higher phosphate concentrations did not improve these effects.

The pH* at 50 mM phosphate was varied from 2.0 to 3.5. Lower apparent pH* gave the

best results with good peak shape and some retention of CAF. A pH* of 2.5 was chosen because resolution was similar to that with pH* 2.0, but useful column life may be longer than with pH* 2.0. Reducing acetonitrile concentration over a range of 25–5% yielded good retention for CAF and PAR at about 5%, but under these conditions ASC was not retained. The peaks corresponding to CHL and DEX became broad eluting within 30 min at a flow rate of 1 ml min^{-1}.

To retain ASC, the basically charged ion pairing agent tetrabutylammonium hydrogen sulphate was added to the mobile phase over a range of 20–125 mM in the presence of 1 mM PSA. The PSA could not be eliminated because at least 1 mM was required to achieve good peak shape with CHL and DEX, but higher concentrations (5–25 mM) were no better than 1 mM. As a result, a mixed ion pairing mobile phase was necessary. Increasing TBA in presence of PSA yielded better peak shape and shorter retention times with good resolution for all peaks. With higher TBA concentrations, ASC was retained whilst the other compounds eluted more rapidly. DEX retention time was strongly affected by the higher TBA concentrations. It increased from 9.8 min at 125 mM TBA to 17.6 min at 20 mM TBA. The capacity factor, k', for DEX decreased two-fold at 125 mM TBA compared to 20 mM. The peak shapes of the basic compounds CAF, CHL and especially DEX were much improved. An analysis of k' for all components demonstrated that CAF which was retained more than PAR at 40 mM TBA (PAR 3.5 min, CAF 4.2 min) was less retained than PAR at 125 mM TBA (CAF 2.8 min, PAR 3.1 min). This indicated a selective effect on the basic drugs (Fig. 1). Because of the symmetrical, quaternary amine chemical structure of TBA $[N^+(C_4H_9)_4]$ and its short alkyl chain length (C4), micelle formation was unlikely even at this relatively high concentration (125 mM). The primary effect of the TBA was to reduce peak tailing and despite the end-capping, tailing of the basic compounds was a problem without relatively large concentrations of the TBA. The newly available base deactivated columns might reduce the need for high TBA concentrations by reducing binding to bare silica. The PSA acted primarily to ion pair with the basic compounds. Higher concentrations of PSA on the order of 25–30 mM appeared to interact to an extent with the TBA

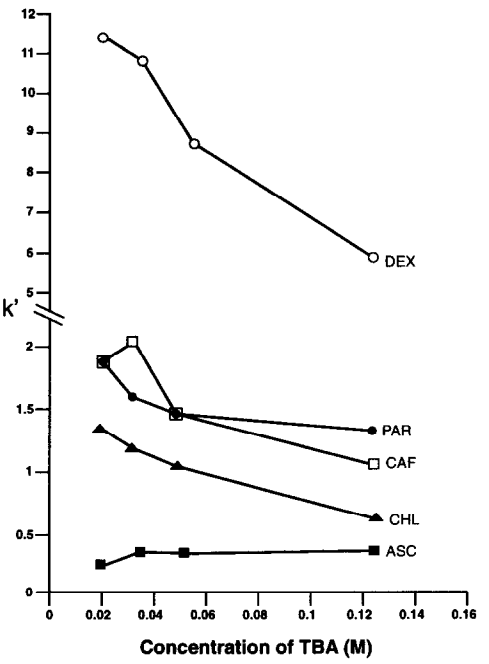

Figure 1
Relationship of the capacity factor, k', to TBA concentration under standard conditions for the five Frenadol active drug substances.

and thereby reducing its effectiveness. The ratio of TBA to PSA was important and considerable experimentation was needed to determine the final ratio.

An end-capped, 5 μm particle size, phenyl column was employed to reduce the binding to the silica backbone for the basic compounds that occurred with the μBondapak column, to increase the number of available theoretical plates and to prolong useful column life. Temperature was varied over a range of 25–50°C. At 40°C the separation was optimum with a shorter retention time for all components but good overall resolution. Finally, the flow rate was increased to 2 ml min^{-1} in order to reduce the assay run time without deleterious effects on resolution.

Figure 2 shows the final separation conditions at two wavelengths, 210 and 290 nm. The peak areas were largest for all compounds at 210 nm, but the absorbance for the 150% level (1.95 mg ml^{-1}) of PAR at 210 nm exceeded the linear range of the detector (2.0 AU) at the level necessary for detection of the other components which were present in much lower concentrations, e.g. CHL = 0.008 mg ml^{-1}. The PAR peak was within detection range at 290 nm (<2.0 AU). Moreover, citric acid in the sachets interfered with

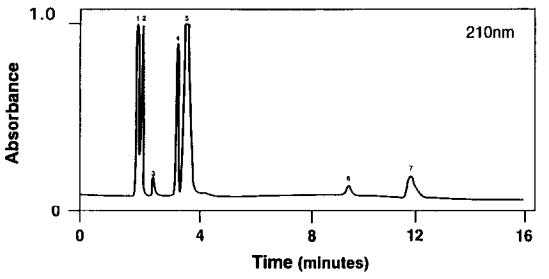

Figure 2
Separation of Frenadol sachet with detection at 210 nm. Peaks were identified as: 1, ASC; 2, citric acid; 3, CHL; 4, CAF; 5, PAR; 6, maleic acid; and 7, DEX.

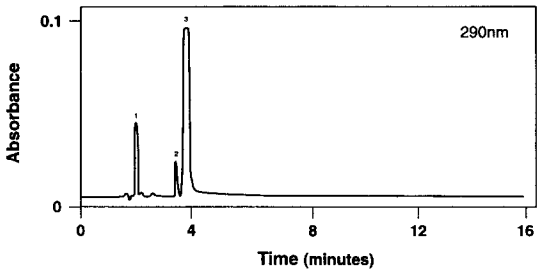

Figure 3
Separation of Frenadol sachet with detection at 290 nm. Peaks were identified as: 1, ASC; 2, CAF; and 3, PAR.

the ASC peak at 210 nm but not at 290 nm. Both PAR and ASC were routinely assayed simultaneously with the other components by dual detection at 210 and 290 nm. CAF could also be assayed at 290 nm, but this is not done routinely because peak areas were larger and precision better at 210 nm. The method was validated by determining PAR and ASC at 290 nm, while CHL, CAF and DEX were determined at 210 nm. Retention times for the actives were ASC, 1.9 min; CHL, 2.4 min; CAF, 3.1 min; PAR, 3.5 min; and DEX, 11.3 min.

Factors which quantitatively describe the final assay conditions used for validation studies were determined as required in Merck and USP validation protocols [5]. These factors must be considered by analysts using the method for system suitability requirements as required by the Food and Drug Administration (FDA). A key factor in the separation was the resolution between CAF and PAR. To ensure accurate assay, the resolution, R_s, between CAF and PAR must be greater than 1.0. In addition, ASC must be sufficiently retained to separate it from the solvent peak. Although the end-capped column and the high TBA content of the mobile phase reduced tailing of CHL and DEX, some tailing remained with T factors of 1.8 and 1.6, respectively. Tailing can be controlled by modifying the TBA concentrations. The number of theoretical plates was in excess of 3000 m^{-1} for all the peaks indicating high efficiency. The minimum amounts of the active drug substances that could be quantified were 0.1% of the 100% label claim (see above) with 210 nm detection and 1% with 290 nm detection. Minimum detectability of all the actives was approximately 10 ng.

Method validation

Placebo assays. Spiked placebo assays (Table 1) were used to determine the accuracy, precision and linearity of the Frenadol method. The individual excipient constituents of sachets

Table 1
Statistical analysis of spiked placebo assays

Parameter	ASC	CHL	CAF	PAR	DEX
Accuracy* (%)	99.6	99.9	100.6	100.0	100.5
Bias† (%)	−0.4	−0.1	0.6	0	0.5
Precision‡ (%)	2.0	1.8	1.8	0.4	1.6
Linearity§: slope	0.99	1.00	1.00	1.00	1.01
Coefficient of determination	0.9994	0.9994	0.9995	0.9999	0.9996

*Derived from spiked placebo validation assays with 100 injections for 50 samples by two independent analysts on different days. Defined as
$\frac{[\text{Found}]}{[\text{Theory}]} \times 100\%$ ($n = 20$).
†Defined as
$\frac{[\text{Found}] - [\text{Theory}]}{[\text{Theory}]} \times 100\%$ ($n = 20$).
‡RSD ($n = 20$).
§$n = 20$.

were weighed, mixed and spiked in the same quantities as the Frenadol sachets for the 100% level (see above). Bracketing levels of 150, 125, 75 and 50% were also assayed. A statistical summary of the results is shown in Table 1. Accuracies were well within Merck's internal specification limits of ±2% with recoveries ranging from 99.6 to 100.6% of the amount of active ingredient spiked into the placebo. The bias (mean less theory) showed only minor variations in recovery at each level with 0.6% the maximum variation observed. The 2 sigma (2σ) or 95% confidence intervals indicated precision that met internal specifications at 2% or less. The slopes varied only 0.01 from 1.00 which indicated linearity within the specification of 0.98–1.02. In addition, all the coefficients of determination or R^2 were in excess of 0.999. In summary, the data (Table 1) indicated that the method was precise, linear and accurate over the range tested and met all Merck internal specifiations (±2% RSD) for a validated HPLC method within and between days. Samples were stable for at least 24 h in solution. The method complied with USP [5] and EC validation guidelines [6].

Ruggedness testing. As an indication of the ruggedness and applicability of the assay to actual Frenadol samples, composite assays (mixed sachets) for Frenadol were performed by two different analysts on two different instruments with two separate mobile phase preparations. The data shown in Table 2 are a summary of the analysis of 12 samples. The two sets of results were found to be equivalent within experimental errors.

Dose uniformity assays were performed on individual sachets by two analysts on two different instruments with two separate mobile phase preparations. The results (Table 3), which originated from the analysis of 60 samples, are again identical within experimental errors. Based on these studies, it is clear that transfer of the methods to quality control laboratories would be uncomplicated.

Table 2
Composite assays of three Frenadol lots*

Sample	Recovery (%)†	
	Analyst no. 1	Analyst no. 2
ASC	102.1 ± 0.6	101.5 ± 1.0
CHL	100.9 ± 1.1	99.7 ± 1.0
CAF	98.6 ± 0.7	99.1 ± 0.7
PAR	98.4 ± 1.0	98.4 ± 0.6
DEX	101.1 ± 0.2	101.1 ± 1.4

*Data calculated from composite assay data with 12 samples and 24 assays by two independent analysts. Data is presented as mean% ± RSD% recovered from composite sachets compared to a standard.

†Defined as $\frac{[\text{Found}]}{[\text{Label}]} \times 100\%$ ($n = 12$).

Conclusions

The five active drug substances of Frenadol, a cold medication, which had previously been assayed with five separate methods can now be assayed simultaneously using a single liquid chromatographic (LC) method. The method separates ASC, CHL, maleic acid, CAF, citric acid, PAR and DEX in 15 min. An extensive validation study, which included spiked placebo experiments and ruggedness testing, met all USP [5] and EC [6] validation requirements. The method was found to be precise, reproducible, linear, accurate and rugged.

Table 3
Dose uniformity assays for Frenadol sachets*

Compound	Recovery (%)†					
	Batch no. 1		Batch no. 2		Batch no. 3	
	Anal. no. 1,	Anal. no. 2	Anal. no. 1,	Anal. no. 2	Anal. no. 1,	Anal. no. 2
ASC	102.3,	100.5	100.3,	98.9	100.1,	99.5
CHL	100.6,	100.8	101.2,	102.2	101.3,	99.9
CAF	98.5,	99.1	98.5,	99.6	98.4,	100.4
PAR	98.3,	98.2	97.8,	98.1	99.5,	99.8
DEX	100.8,	101.5	100.2,	101.2	100.4,	100.1

*Data calculated from dose uniformity assays of 60 samples and 120 injections performed by two independent analysts (Anal. no. 1, no. 2). Data is presented as mean% recovered from sachets.

†Defined as $\frac{[\text{Found}]}{[\text{Label}]} \times 100\%$ ($n = 20$).

References

[1] G.T. Greco, *Drug Devel. Indust. Pharm.* **10**, 19–30 (1984).
[2] V. Das Gupta and A.R. Heble, *J. Pharm. Sci.* **11**, 1553–1556 (1984).
[3] V. Das Gupta and J.T. Jacob, *Drug Devel. Indust. Pharm.* **13**, 113–126 (1987).
[4] T.A. Biemer, *J. Chrom.* **410**, 206–210 (1987).
[5] *United States Pharmacopeia XXI*, pp. 1174–1178 and 1226–1230. The United States Pharmacopeial Convention, Rockville, MD (1985).
[6] *European Pharmacopoeia*, v.6.20.4. European Pharmacopoeial Convention, P. Arens, Chairman (1987).

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