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A fast and efficient determination of amines and preservatives in cough and cold liquid and suspension formulations using a single isocratic ion-pairing high power liquid chromatography method

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Abstract

A single, highly selective ion-pairing reverse phase-high power liquid chromatography (RP-HPLC) method has been developed for the determination of amines and preservatives in a wide range of Tylenol® liquid and suspension liquid products. As with many OTC products, the challenge is to quantitatively extract the analytes from difficult matrices and specifically analyze them in the presence of various excipients and flavors. Historically, separate analytical methods were used for each class of analytes (acids, bases and neutral compounds). In this method a mobile phase consisting of a buffered ion-pairing agent with acetonitrile, methanol and tetrahydrofuran was used to separate the charged amines from neutral and acidic compounds on a Phenomenex LUNA C8(2) 75 × 4.6 mm i.d. analytical column with a 3-μm particle size. The analytes include acids (benzoic acid), bases (pseudoephedrine, chlorpheniramine, dextromethorphan, doxylamine and diphenhydramine) and a neutral compound (butylparaben). The effects of pH, the chain length of the ion-pairing reagent, ionic strength and organic modifiers on the separation are discussed. The method is linear from 15 to 150% of the target amounts. The optimized method proves to be specific, robust and accurate for the analysis of the compounds.

Keywords: Ion-pairing RP-HPLC; Antitussive; Antihistamine; Nasal decongestant; Preservatives

1. Introduction

Cough and cold remedies have been available to the consumer as over-the-counter (OTC) products for several decades. Since the complete prevention of infections induced by cold viruses does

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not yet exist, there is an overwhelming demand for sinus, cough/cold and allergy OTC medications for the relief of cold symptoms.

These medications are usually some combination of an analgesic (e.g. acetaminophen), antitussive (e.g. dextromethorphan hydrobromide, guaifenesin), an antihistamine (e.g. chlorpheniramine maleate, diphenhydramine hydrochloride or doxylamine succinate) and a nasal decongestant (e.g. pseudoephedrine hydrochloride). Common formulations of these products are liquids or suspensions and therefore require the addition of preservatives (e.g. benzoic acid, butylparaben).

In many cases, for example Tylenol® products, the concentration of acetaminophen is significantly higher than the other actives and preservatives. The presence of different classes of active chemicals (neutral, amines and carboxylic acid) and the disparity in concentration and formulation matrix poses analytical challenges. Typically several methods are used to analyze one mixture. Ion exchange or reverse phase ion-pairing chromatography separates the amines. Acetaminophen and the preservatives are separated using different reverse phase methods. This approach is both time consuming and costly.

Several methods are reported in the literature proposing fast and reliable techniques to separate cold relief ingredients [1–4]. However, a number of these methods are practical only for a few active amines and do not include the preservatives in the separation [5,6]. The work presented in this manuscript describes a rapid and reliable reverse-phase isocratic HPLC method using ion-pairing chromatography to separate five of the most commonly used active ingredients found in OTC cough and cold liquid and suspension medications as well as the preservatives benzoic acid and butylparaben.

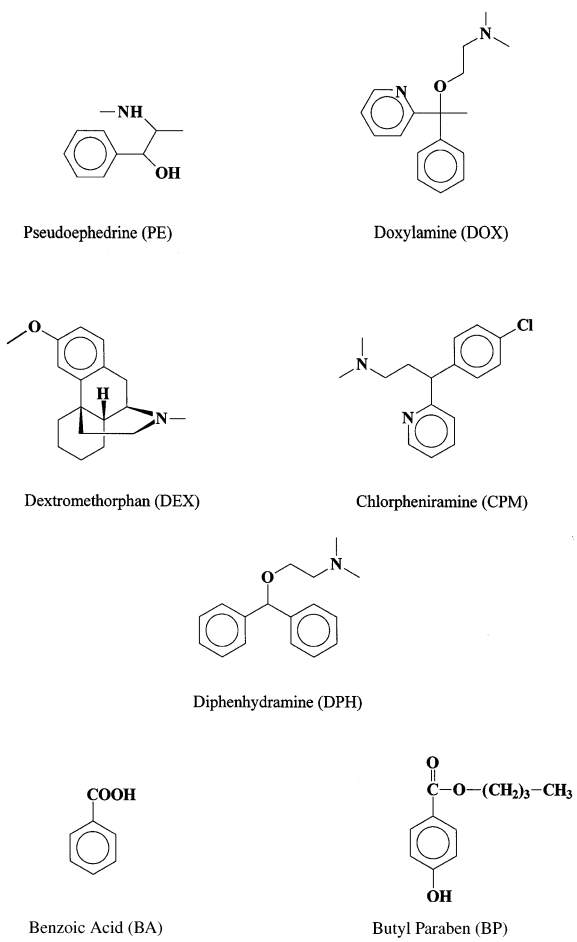

Fig. 1. Chemical structure of compounds.

2. Experimental

2.1. Materials

Working reference standards of acetaminophen, benzoic acid, pseudoephedrine HCl, doxylamine succinate, butylparaben, chlorpheniramine maleate, dextromethorphan hydrobromide and diphenhydramine hydrochloride were supplied by McNeil Consumer Healthcare, Fort Washington, PA. The structures of these compounds are shown in Fig. 1. Methanol, acetonitrile and tetrahydrofuran (THF) that were used were HPLC grade and purchased from EM Scientific (Gibbstown, NJ). In-house Milli-Q water was used to prepare all buffered and standard solutions. All other reagents were of analytical grade and were purchased from VWR Scientific (Bridgeport, NJ) and Fisher Scientific (Fair Lawn, NJ).

2.2. HPLC system

The chromatographic system consisted of a Waters (Milford, MA) Model 600 controller

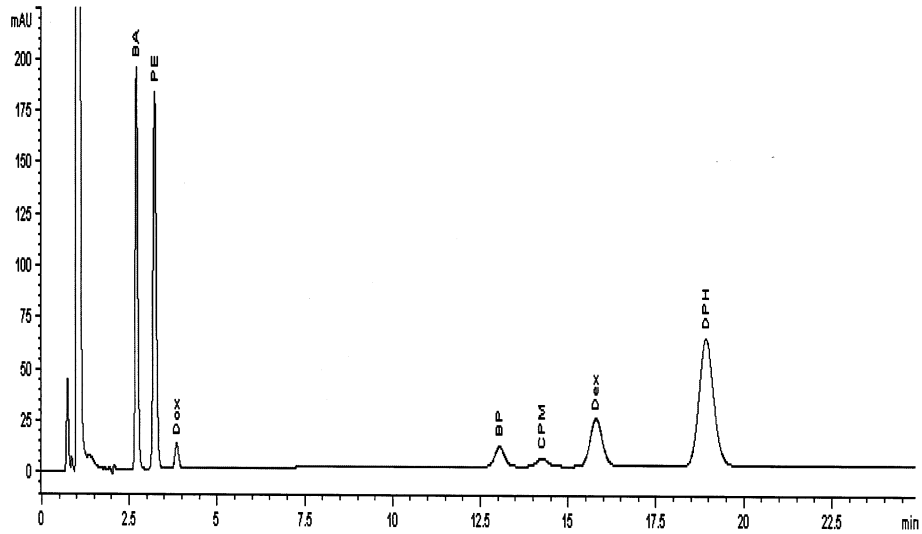

Fig. 2. Representative chromatogram of standard solution; BA, benzoic acid 90.0 μg/ml; PE, pseudoephedrine 150.0 μg/ml; Dox, doxylamine 12.5 μg/ml; BP, butylparaben 12.5 μg/ml; CPM, chlorpheniramine 10.0 μg/ml; Dex, dextromethorphan 50.0 μg/ml; DPH, diphenhydramine 12.5 μg/ml. Column: phenomenex LUNA C8(2) 75 × 4.6 mm 3 μ; mobile phase: 0.1 M NaH_2PO_4 buffer (pH 2.1) containing 50 mM 1-octanesulfonic acid, sodium salt–acetonitrile–methanol–tetrahydrofuran (51:5:42:2, v/v/v/v); flow rate: 1 ml/min; detector; 214 nm.

Table 1
System suitability

No.	Parameters	BA	PE	DOX	BP	CPM	DEX	DPH
1	Resolution factor[a]	–	3.65	3.89	21.10	2.56	3.00	3.61
2	Tailing factor	1.21	1.18	1.15	1.13	1.11	1.11	1.16
3	%RSD for seven injections	0.18	0.17	0.22	0.17	0.14	0.13	0.51

[a] The resolution factor is calculated between each peak and its nearest preceding neighbor.

Table 2
Linear analytical response statistical summary for all analytes using peak areas[a]

Analyte	Intercept	Standard error of intercept	Slope	Standard error of slope
BA	35362.0	6920.8	19901.0	82.9
BP	−1301.1	602.5	31828	52.0
CPM	−1985.9	498.7	16666.8	54.2
DEX	967.6	871.1	12845.7	19.2
DOX	−85.8	319.8	13146.5	27.9
DPH	19601.3	5823.8	26747.0	49.3
PE	84617.2	15483.7	12715.5	113.5

[a] BA, benzoic acid; PE, pseudoephedrine; DOX, doxylamine; BP, butylparaben; CPM, chlorpheniramine; DEX, dextromethorphan; DPH, diphenhydramine. Detector wavelength: 214 nm. Seven concentration levels for each analyte except BA and BP (5). Triplicate injections of each concentration level.

equipped with a Model 610 isocratic fluid unit pump. All samples were injected (10 μl) using a Waters 717 Plus autosampler. Elutions of all analytes were monitored at 214 nm by using a Waters 486 tunable absorbance detector containing a 10 μl flow cell. Each chromatogram was analyzed and integrated using the Waters ExpertEase Suitability Software version 3.2 or HP Chemstation V.6.

2.3. Chromatographic conditions

The HPLC separations were achieved using a Phenomenex (Torrance, CA) LUNA C8(2) analytical column, 75 × 4.6 mm i.d., with a 3-μm particle size. The aqueous component of the mobile phase was a 0.1 M NaH_2PO_4 buffer containing 0.05 M HPLC grade 1-octanesulfonic acid sodium salt, adjusted to pH 2.1 with 6 M HCl prior to diluting to volume. The mobile phase for the cough and cold formulations consisted of a buffer–acetonitrile–methanol–tetrahydrofuran (51:5:42:2, v/v/v/v) blend that was premixed and filtered through a 0.45 μm nylon membrane filter and vacuum degassed with sonication prior to column equilibration.

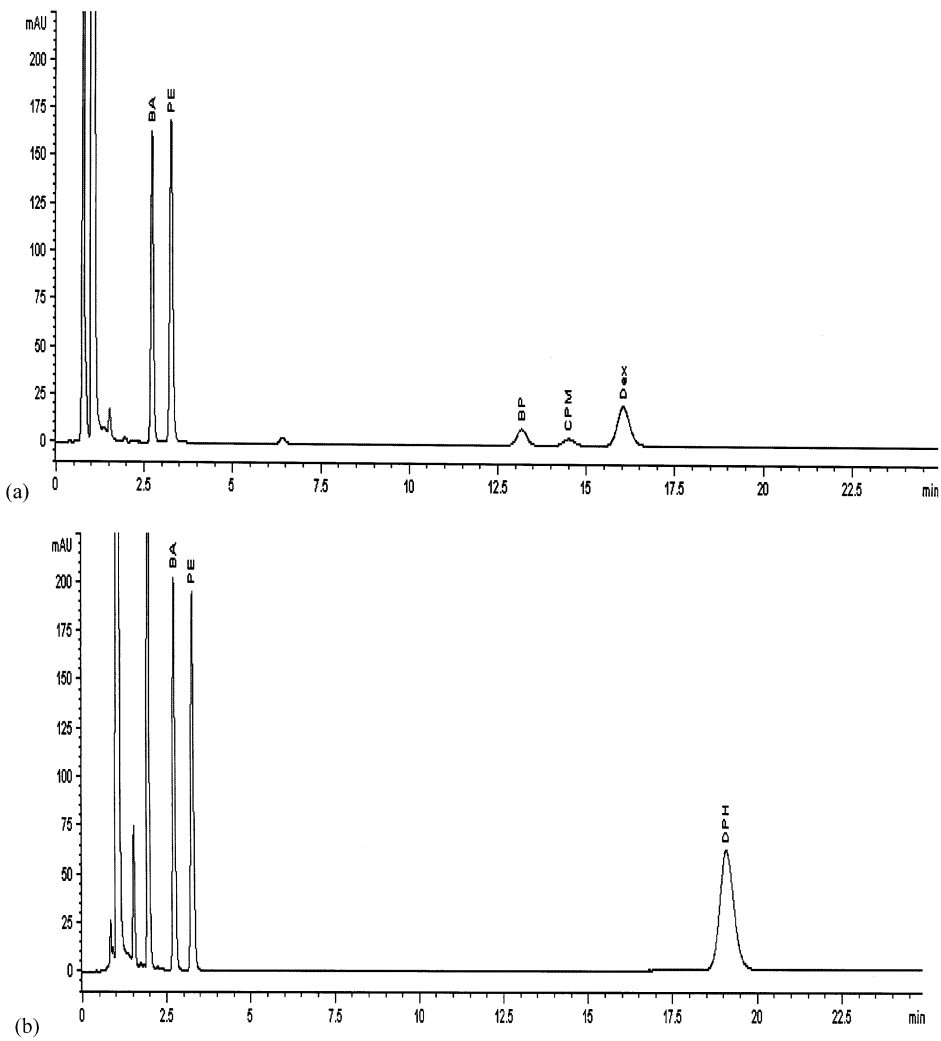

Fig. 3. (a) Representative chromatogram of Children's Tylenol Flu suspension liquid. (b) Representative chromatogram of Children's Tylenol Allergy-D liquid.

Chromatography was performed at ambient conditions with a flow rate of 1.0 ml/min.

2.4. Sample and standard preparation

2.4.1. Preparation of suspension liquid samples

The cough and cold formulas that are liquid suspensions require the precipitation of the cellulose and gums prior to injection to prevent column build up and high backpressure. The isolation of analytes from cellulose and gums was achieved by diluting 6.4 g (5.0 ml) of sample into 20 ml of 0.2 M NaH_2PO_4, pH 2.0 buffer solution in a 100-ml volumetric flask. This solution was mixed using a mechanical flat bed shaker. Next, about 60 ml of methanol was added to the sample solution with additional shaking. The addition of methanol ensured the precipitation of the gums. The sample solution was left to cool at room temperature before diluting to volume with methanol. The resulting sample mixture was centrifuged and the supernatant used for HPLC analysis.

2.4.2. Preparation of suspension liquid standards

A stock solution of all analytes, excluding acetaminophen, was prepared at a concentration that was ten times greater than the label claim of the products being analyzed. All standard solutions were prepared with methanol–water mixture (80:20, v/v). A 10-ml aliquot was transferred to a 100-ml volumetric flask containing 160 mg of acetaminophen. The standard was diluted with 40 ml of the 0.2 M NaH_2PO_4, pH 2.1 buffer and methanol with intermittent shaking using the same procedure described above in the sample preparation.

2.4.3. Preparation of liquid samples

A dosage unit amount was transferred to a volumetric flask and diluted to volume with water. The sample was mixed on a mechanical flat bed shaker for a fixed amount of time to ensure the sample was homogeneous.

2.4.4. Preparation of liquid standards

A 10-ml portion of the stock solution prepared for the suspension liquids was transferred to a 100-ml volumetric containing 160 mg of acetaminophen. The sample was diluted to volume with Milli-Q water and thoroughly mixed.

3. Results and discussion

3.1. Developing the method

3.1.1. Ion-pairing reagents

Many of these OTC products contain flavors and excipients that are for the most part highly polar compounds that must be accounted for when developing the method. It was observed that pseudoephedrine was co-eluting with excipients when its capacity factor (k') is less than the k' for benzoic acid. Several ion-pairing reagents were tried during our preliminary work, ranging from C5–C12 sulfonates. The longer alkyl chain length of the ion-pairing reagent the more retained the amines, however octanesulfonic acid was the only ion-pairing reagent that was able to produce the desirable chromatographic separation. First, the shorter-chained alkyl-sulfonates did not retain pseudoephedrine and doxylamine strongly enough and hence they would precede benzoic acid. Secondly, there was co-elution with pseudoephedrine, doxylamine, the excipients and acetaminophen at or close to the solvent front. Alkyl-sulfonates with chain lengths longer than eight carbons at various concentrations caused the last eluting peak, diphenhydramine, to be strongly retained yielding run times that were impractical for any QC environment.

3.1.2. The counter ion

Ion-pair chromatography, in part, depends on ion exchange phenomena; therefore the ionic strength of the buffer and the nature of the counter ion were also critical for the separation. A decrease in ionic strength leads to an increase in retention for compounds (amines) that interact with the ion-pair reagent. Also, the relative strength of the cation will affect the retention. More positively charged cations reduce sample retention [7]. Potassium from KH_2PO_4 proved to be too strong of a cation for the separation of the critical pair, pseudoephedrine and benzoic acid, to

achieve a resolution of more than 0.8. Sodium was substituted for potassium by using $NaH_2PO_4H_2O$. The concentration of 0.1 M $NaH_2PO_4.H_2O$ was the optimum concentration for the separation. A concentration less than 0.1 M $NaH_2PO_4.H_2O$ increased the retention of chlorpheniramine resulting in the co-elution of chlorpheniramine and dextromethorphan. A concentration exceeding 0.1 M $NaH_2PO_4.H_2O$ resulted in a decreased resolution between the early eluting analytes (e.g. excipients, benzoic acid and pseudoephedrine) as well as the co-elution of butylparaben and chlorpheniramine. Chlorpheniramine was the most sensitive to changes in ionic strength, ion-pairing reagent and pH. This is attributed to its two ionizable nitrogens, pyridinium nitrogen at the 2 position (pK_a 3.6) and tertiary amine nitrogen (pK_a 9.1).

3.1.3. Organic solvents

The organic solvents acetonitrile and methanol have different effects on the solvation of the analytes and therefore they also affect the symmetry and selectivity of the peaks. A binary mobile phase of 50% methanol and buffer was originally used to achieve a separation of all seven analytes. This is the minimum concentration of the aqueous portion required to retain the most polar neutral analyte, benzoic acid, off the solvent front. The neutral analyte, butylparaben was retained at more than 30 min. As mentioned, the retention of the charged analytes can be altered by adjusting the buffer and ion-pairing parameters. The addition of acetonitrile to the mobile phase decreased the retention of butylparaben while maintaining the retention of benzoic acid. An acetonitrile concentration of greater than 5% caused asymmetrical peak shapes of the amines. A mobile phase of 5% acetonitrile, 44% methanol and 51% buffer proved to have the best resolution between all seven analytes and maintain symmetrical peaks. The optimum solvent conditions using acetonitrile, methanol and buffer however did not produce a separation that was satisfactory with a reasonable overall runtime. Substitution of 1% methanol with tetrahydrofuran did not decrease the overall run time appreciably. Incorporation of 2% tetrahydrofuran into the mobile had a pronounced effect on the overall run time while maintaining a resolution factor (R_s) > 2.0 for all analytes. For example, replacing 2% methanol with tetrahydrofuran while keeping all other conditions constant, decreased the retention of dextromethorphan from 25.2 min to 15.3 min. Concentration of THF at 3% level caused the co-elution of benzoic acid and pseudoephedrine near the solvent front. The incorporation of just 2% tetrahydrofuran into the mobile phase is extremely advantageous as it reduces the overall runtime drastically. Fig. 2 shows an example of a standard prepared with a concentration of 100% of the target concentrations of each analyte. The incorporation of THF into the mobile phase changed the critical pair for the separation to chlorpheniramine and butylparaben.

3.2. System suitability

The system suitability was determined by making seven replicate injections and analyzing each solute for their peak area, resolution and peak-tailing factor. The system suitability requirements for the amines and preservatives was a %RSD for peak area less than 2.0, a peak tailing factor less than 2.0 and an R_s greater than 2.0 between adjacent peaks for all analytes. This method met these requirements. The results are shown in Table 1. The critical pair for this separation was chlorpheniramine and butylparaben with R_s of 2.56.

3.3. Linearity

The range of linearity using this method was 15–150% of the target concentration of all seven analytes. Standard curves were plotted using peak area as well as peak height at the wavelengths of 214 and 222 nm versus solute concentration. The method is linear from 13.5–135.2 μg/ml and 1.9–18.8 μg/ml for the preservatives benzoic acid and butylparaben, respectively. Pseudoephedrine.HCl (22.5–225.0 μg/ml), chlorpheniramine maleate (1.5–15.2 μg/ml), doxylamine succinate (1.9–18.8 μg/ml), dextromethorphan.HBr (7.5–75.0 μg/ml) and diphenhydramine.HCl (19.5–195.0 μg/ml) were also linear over these concentration ranges.

Each compound had R^2 values of 0.999 or greater. Statistical summaries of linear response of concentration (μg/ml) versus peak area for all analytes are shown in Table 2.

3.4. Assay

Six different cough and cold formulas were analyzed with the proposed method and the concentrations of each compound were determined and compared to the label amount. Representative chromatograms are shown in Fig. 3. The %RSD was less than 1.2 for pseudoephedrine and less than 1.0 for all other analytes. The % recovery of all analytes from these formulas ranged from 92.2 (butylparaben) to 103.0 (pseudoephedrine). The low %RSD values calculated for each compound and recoveries of more than 92% indicate that this method is reliable and could be applied to a variety of cold and flu relief products.

3.5. Accuracy and precision

Triplicate solutions of the amines and the preservatives were prepared at the 50, 75, 100, 125 and 150% of the nominal concentrations in the products tested. Each solution was spiked with a fixed amount of the active ingredients and the preservatives. The concentration of acetaminophen was held constant (1.6 mg/ml). The amounts recovered were in the 98.4–102.2% range of the expected values.

3.6. Reproducibility

The 100% standard was injected 30 times successively and the retention times and peak areas were compared. The %RSD of peak areas was less than 1.0% for the amines and the preservatives showing that the method is reproducible.

4. Conclusions

A simple, accurate and reproducible reverse phase HPLC method has been developed for simultaneous assay of a variety of active ingredients and the preservatives found in the most commonly used cough and cold liquid and suspension formulas. Analysts can now conserve time and costs by analyzing several active ingredients and the preservatives in cold, allergy and sinus liquid medications using one instrument, column and mobile phase. This method could be applied to numerous liquid and suspension liquid cough and cold/flu formulations. The method is rapid and has been shown to be reproducible and reliable.

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