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Validation of improved methods for high-performance liquid chromatograpic determination of phenylpropanolamine, dextromethorphan, guaifenesin and sodium benzoate in a cough-cold formulation

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ABSTRACT

Improved methods for the HPLC determination of phenylpropanolamine · HCl, dextromethorphan · HBr, guaifenesin and sodium benzoate in an oral liquid cough-cold product have been validated. The methods were shown to be accurate, precise, selective and rugged as would be required for regulatory submission and more efficient in terms of sample and standard preparation than previous methods. Phenylpropanolamine and dextromethorphan were analyzed simultaneously on a silica based SCX column using a buffered mobile phase with detection at 263 nm, while sodium benzoate and guaifenesin were measured together using a reversed-phase C_{18} column, an aqueous–organic mobile phase of controlled ionic strength and detection at 273 nm. A column heater was also used, set at 40°C and 35°C for these respective determinations.

INTRODUCTION

The quantitative analysis of components in liquid oral cough-cold products has been previously accomplished by diverse methods from spectrophotometric to chromatographic. Previous HPLC methods have measured these constituents either individually or in combination. LC assays that have been reported on individual components of interest to the present investigation include: phenylpropanolamine (PPA) [1–6], dextromethorphan (DM) [7–11], sodium benzoate (B) [12,13] and guaifenesin (G) [14]. These methods commonly provided determinations for additional active components not of interest to the present cough-cold product, Naldecon DX Pediatric Syrup (Bristol–Myers Squibb, Evansville, IN, USA). Simultaneous HPLC assays have been described on phenylpropanolamine-sodium benzoate–guaifenesin [15,16], phenylpropanolamine–dextromethorphan [17], phenylpropanolamine–guaifenesin [18] and dextromethorphan–guaifenesin [19],

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usually along with other components. Among these latter studies, two were internal standard methods using C_{18} columns [18,19], one was an internal standard, phenyl column method [16] and one was an external standard method which used a C_{18} column along with a standard addition method to measure accuracy [17]. The final simultaneous assay method used external standards with accuracy validated by means of spiked placebos employing a C_8 column with an ion-pairing mobile phase [15]. It was desired to validate two more efficient (in terms of sample and standard preparation) external standard methods in the present study, one of which could be used to assay guaifenesin and sodium benzoate and the other to assay phenylpropanolamine · HCl and dextromethorphan · HBr in the cough-cold preparation, simultaneously.

Reagents and drugs

HPLC-grade solvents for chromatography included acetonitrile and methanol from J.T. Baker (Phillipsburg, NJ, USA) and reagent-grade water, processed at Lancaster Labs (Lancaster, PA, USA). Reagent-grade glacial acetic acid, hydrochloric acid (12 *M*), sodium hydroxide (pellets) and hydrogen peroxide (30%) were from Fisher Scientific (Fair Lawn, NJ, USA) while ammonium dihydrogen phosphate was from J.T. Baker and diethylamine was from Aldrich (Milwaukee, WI, USA). Drugs used in the investigation include guaifenesin (lot G-3), dextromethorphan · HBr (lot H) and phenylpropanolamine · HCl (lot H) from the United States Pharmacopeial Convention (Rockville, MD, USA) and sodium benzoate (lot KX06814HW) from Aldrich. The drug product Naldecon DX Pediatric Syrup (lot MHM44) and the product placebo (Lot E91L047 549-GN-152) were from Bristol–Myers Squibb.

Instrumentation

HPLC analysis was conducted using a modular HP 1050 system (Hewlett-Packard, Palo Alto, CA, USA) consisting of a pump run at 2.0 ml/min for the PPA–DM assay and 1.3 ml/min for the B-G assay. The variable-wavelength UV detector was set at 263 nm for the PPA–DM assay and 273 nm for the B–G assay. The autosampler injected 20 μl in the PPA–DM assay and 25 μl in the B–G assay. Columns utilized included Waters (Milford, MA, USA) μBondapak C_{18}, 10 μm particle size, 30 cm × 3.9 mm for the B–G assay and Whatman (Clifton, NJ, USA) PXS SCX, 10 μm particle size, 25 cm × 4.6 mm for the PPA–DM assay. A Model CH-30 column heater was used from Flatron Systems, Eppendorf N.A. (Madison, WI, USA), while chromatographic data were acquired and analyzed using a Chrom Perfect system (Justice Innovations, Palo Alto, CA, USA).

Mobile phases

A buffered aqueous–organic mixture consisting of 0.1 *M* $(NH_4)(H_2PO_4)$–MeOH (30:70, v/v) with an apparent pH of 6.2 was used in the PPA–DM assay with the column temperature controlled at 40°C while the B–G assay employed water–diethylamine–glacial acetic acid–acetonitrile (739:1:10:250, v/v, apparent pH 4.1) with the column temperature controlled at 35°C.

Procedure

Standard preparation

Phenylpropanolamine–dextromethorphan assay. A mixed standard solution was prepared containing 1.25 mg/ml, 1.00 mg/ml and 1.00 mg/ml of phenylpropanolamine · HCl, dextromethorphan · HBr and sodium benzoate reference standards, respectively in water. A 10-ml volume of this solution was added to a 50-ml volumetric flask containing 200.0 mg of guaifenesin reference standard which was then diluted to volume with water to give the mixed working standard for the PPA–DM assays.

Guaifenesin–sodium benzoate assay. A 5-ml volume of the diluted mixed standard from the phenylpropanolamine–dextromethorphan assay above was further diluted to 100.0 ml with water to give the mixed standard for the B–G assay.

Sample preparation

Phenylpropanolamine–dextromethorphan assay. A 10-ml volume of sample was transferred to a 50-ml volumetric flask using a TC ('to contain') pipet which was rinsed several times with water. The rinsings were added to the flask and it was diluted to volume with water and mixed.

Guaifenesin–sodium benzoate assay. A 5-ml volume of the sample preparation from the phenylpropanolamine–dextromethorphan assay above was further diluted to 100.0 ml with water to give the final sample dilution for the B–G assay.

System suitability

Phenylpropanolamine–dextromethorphan assay. System precision was shown by 6 replicate injections of the mixed working standard which must give an R.S.D. of ≤2.0% by peak area. The resolution factor between the DM and PPA peaks must be ≥6.0. The tailing factor for both PPA and DM peaks must be ≤2.0.

Guaifenesin–sodium benzoate assay. Precision was shown by 6 replicate injections of the final mixed standard which must give an R.S.D. of ≤2.0% by peak area. The resolution factor between guaifenesin and sodium benzoate must be ≥4.0. The tailing factor for both guaifenesin and benzoate must be ≤2.0.

Accuracy —linearity of recovery

Phenylpropanolamine–dextromethorphan assay. Samples were prepared at 80, 100 and 120% of the label sample amounts of PPA, DM, B and G in triplicate containing an appropriate volume of the placebo solution and were assayed by the described method.

Guaifenesin–sodium benzoate assay. The synthetic samples prepared above containing placebo plus 80, 100 or 120% of sodium benzoate, guaifenesin, phenylpropanolamine · HCl and dextromethorphan · HBr, were diluted with water to the prescribed volume in triplicate and were assayed.

Precision

Phenylpropanolamine–dextromethorphan assay. System precision measurements were made from 10 replicate injections of a single sample preparation. Method precision —ruggedness was measured by assaying six sample preparations from a composite by two analysts in different labs each using new standards and mobile phase.

Guaifenesin–sodium benzoate assay. System and method precision were determined as for the PPA–DM assay.

Selectivity

Phenylpropanolamine–dextromethorphan assay. Method selectivity was shown by stressing samples of the product (containing the three actives and the benzoate preservative) which were diluted 10 in 50 with water by means of heat (reflux, 24 h), light (Rayonet Photochemical Reactor Model RMR-500, Southern New England Ultraviolet Co., Hamden, CT, USA, 5 days), peroxide (5 ml 5% H_2O_2 brought to reflux and cooled), acid (5 ml 1 *M* HCl brought to reflux and cooled, neutralized and diluted to volume) and base (5 ml 1 *M* NaOH brought to reflux and cooled, neutralized and diluted to volume). In addition placebos were evaluated chromatographically to ensure there was no interference in the assay.

Guaifenesin–sodium benzoate assay. Method selectivity was tested using the same stressed (heat, light, peroxide, acid and base) diluted solutions as for the PPA–DM assay as well as the corresponding placebo.

RESULTS AND DISCUSSION

Drug product stability studies require potency assay validation data for submission to regulatory authorities in support of each method. The validation minimally requires proof of accuracy (linearity of recovery from spiked placebos), precision (system and method) and selectivity (through stressed drug substance or drug product) along with some indication of ruggedness (interlab, interanalyst or interday adequacy of performance). In order to facilitate rapid sample preparation and analysis of multicomponent products, it would be desirable to combine the procedures for as many components as possible into simultaneous methods. For these multi-

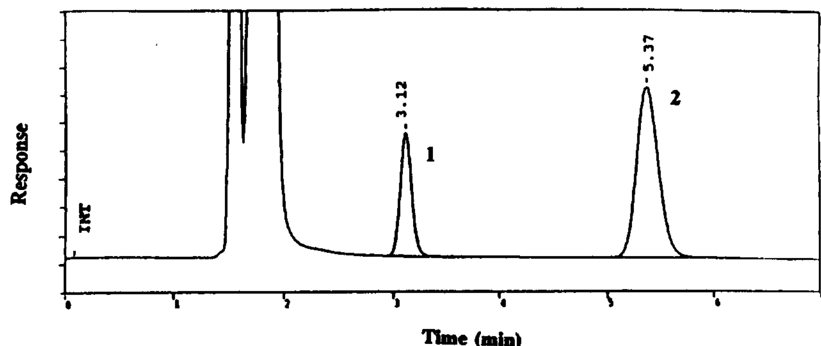

Fig. 1. Chromatogram (peak response *vs.* time) of a standard preparation for the PPA–DM assay showing peaks for phenylpropanolamine (1) and dextromethorphan (2) at 3.1 and 5.4 min, respectively.

component products, methods validation requirements are complicated by the necessity of validating the assay for each component knowing the possibility that degradation products from one component or excipient could interfere with the analysis of another component.

Method selectivity

The method for PPA and DM analysis used a 10-μm 25-cm SCX column with an ammonium phosphate monobasic buffered mobile phase which would produce ionizing conditions at the apparent pH of 6.2 for both bases phenylpropanolamine and dextromethorphan with respective pK_a values of 9.4 and 8.2. The chromatogram of a standard preparation is shown in Fig. 1 with PPA showing the lower retention of the two compounds (3.1 min). The large solvent front peak from 1.5 to 2.5 minutes also contains the other components guaifenesin and benzoate, as would be seen in placebo chromatograms using this method. When the drug product was stressed with heat, light, peroxide, acid or base, no interference was seen with the PPA or DM peaks. Fig. 2, for example, shows the separation of two sets of minor light-induced degradation products at 2.8 and 4.3 min, well removed from the main peaks.

A chromatogram of the standard preparation in the B–G method is shown in Fig. 3. The peak seen at 12 min in all chromatograms in the B–G method is dextromethorphan. Selectivity in the reversed-phase method for guaifenesin and sodium benzoate was also demonstrated by lack of interference from degradation products in chromatograms of acid, base, heat, peroxide and light-stressed samples. Fig. 4 is a chromatogram of a peroxide stressed sample showing the stress-

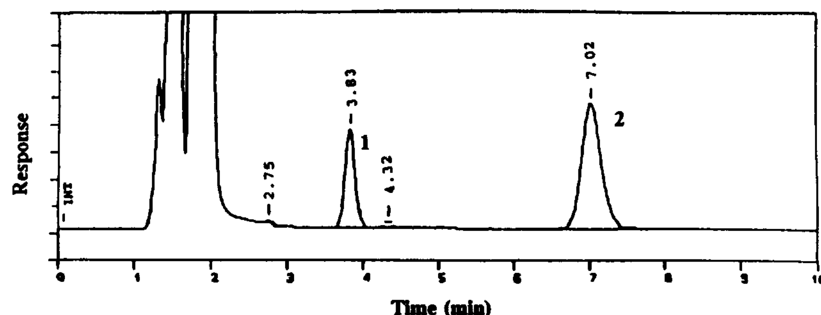

Fig. 2. Chromatogram of a light stressed sample using the PPA–DM assay procedure showing minor stress-induced peaks at 2.8 and 4.3 min. Peaks 1 and 2 as in Fig. 1.

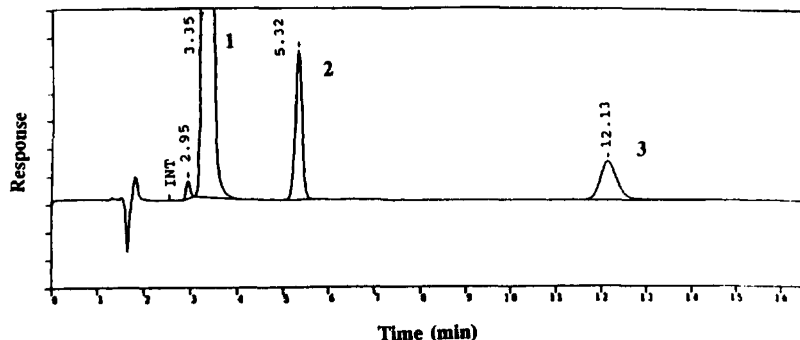

Fig. 3. Chromatogram of a standard preparation for the B–G assay showing peaks for guaifenesin (1) at 3.4 min, benzoate (2) at 5.3 min and dextromethorphan (3) at 12.1 min.

induced degradation products separated from the guaifenesin peak at 3.4 min and the benzoate peak at 5.3 min.

Method accuracy

Accuracy of the described methods is shown by linearity of recovery data for the individual components. Table I gives results for the PPA–DM method with percent recoveries at the 80, 100 and 120% label claim values. Mean recoveries of 100.6 and 100.7% were found for PPA and DM, respectively, along with very acceptable mean R.S.D. recoveries of 1.0 and 0.8%. Similarly for the B–G method with results shown in Table II, mean recoveries of 100.1 and 102.4% were found for guaifenesin and sodium benzoate respectively. Their mean R.S.D. recoveries were also good at 0.4% each. The somewhat elevated benzoate recovery is not considered a systematic error and is in an acceptable range.

Precision and ruggedness

LC method precision measurements can be made to show both system and method reproducibility. In the current study system precision was measured by ten replicate injections of a single sample preparation into both the PPA–DM and the B–G systems. Results of this study shown in Table IIIA, reveal excellent precision with R.S.D. values no greater than 0.3% for each component.

By way of comparison, method precision was demonstrated by analysis of six replicate sample preparations in both the PPA–DM and the B–G systems. Results found in Table IIIB again show high orders of reproducibility in the sample preparation and analysis procedure with R.S.D.

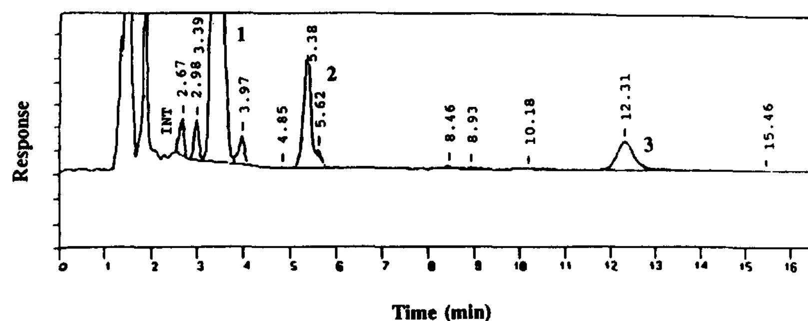

Fig. 4. Chromatogram of a peroxide-stressed sample using the B–G assay procedure. Guaifenesin (1), benzoate (2) and dextromethorphan (3) are seen as in Fig. 3 while the other small integrated peaks are peroxide-induced decomposition products.

TABLE I

LINEARITY OF RECOVERY RESULTS FOR PHENYLPROPANOLAMINE AND DEXTROMETHORPHAN IN SYNTHETIC SAMPLE STUDIES

Phenylpropanolamine · HCl					Dextromethorphan · HBr				
Added (%)	Mean found (%) ($n = 2$)	Recovery (%)	Mean recovery (%)	R.S.D. recovery (%)	Added (%)	Mean found (%) ($n = 2$)	Recovery (%)	Mean recovery (%)	R.S.D. recovery (%)
80.00	80.24	100.2	101.4	1.2	80.16	80.49	100.4	101.2	1.1
79.87	82.13	102.8			79.36	81.32	102.5		
79.87	80.81	101.2			80.00	80.59	100.7		
100.00	99.48	99.5	100.5	0.9	100.20	100.09	99.9	100.5	0.6
99.84	101.07	101.2			99.20	100.36	101.2		
99.84	100.60	100.8			100.00	100.50	100.5		
120.00	118.78	99.0	100.0	0.9	120.24	119.60	99.5	100.3	0.8
119.81	120.61	100.7			119.04	120.28	101.0		
119.81	120.25	100.4			120.00	120.56	100.5		
Mean R.S.D. recovery (%)				1.0					0.8
Slope				0.971					0.985
Intercept				3.419					2.155
Correlation coefficient				0.9987					0.9991

values all less than 0.6% for all four components.

One means of showing method ruggedness (robustness) is to determine variability resulting from a reanalysis of samples by a second analyst using a new standard preparation and new mobile phase with another instrument. Table IIIC shows results for this ruggedness test on the four component cough-cold product. Differences in mean values found for each of the four

TABLE II

LINEARITY OF RECOVERY RESULTS FOR GUAIFENESIN AND SODIUM BENZOATE IN SYNTHETIC SAMPLE STUDIES

Guaifenesin					Sodium benzoate				
Added (%)	Mean found (%) ($n = 2$)	Recovery (%)	Mean recovery (%)	R.S.D. recovery (%)	Added (%)	Mean found (%) ($n = 2$)	Recovery (%)	Mean recovery (%)	R.S.D. recovery (%)
79.6	79.59	100.0	100.2	0.3	79.36	81.47	102.7	103.0	0.4
79.2	79.63	100.5			79.04	81.78	103.5		
81.4	81.37	100.0			79.36	80.67	102.9		
99.8	99.31	99.5	100.1	0.6	99.20	101.26	102.1	102.3	0.3
100.0	100.18	100.2			98.80	101.01	102.2		
98.8	99.35	100.6			99.20	101.85	102.7		
119.6	119.52	99.9	99.9	0.2	119.04	121.10	101.7	102.0	0.4
119.0	119.06	100.1			118.56	120.78	101.9		
120.6	120.20	99.7			119.04	122.06	102.5		
Mean R.S.D. recovery (%)				0.4					0.4
Slope				0.993					1.010
Intercept				0.756					1.319
Correlation coefficient				0.9998					0.9996

TABLE III

PRECISION RESULTS FOR COMPONENTS IN COUGH-COLD PRODUCT

Sample	Phenylpropanolamine · HCl	Dextromethorphan · HBr	Guaifenesin	Sodium benzoate
(A) System precision results found in terms of mg of each component per 5 ml product				
1	6.22	4.97	99.20	5.05
2	6.22	4.97	99.22	5.06
3	6.21	4.96	99.13	5.06
4	6.22	4.96	99.59	5.06
5	6.20	4.95	99.74	5.09
6	6.21	4.96	99.13	5.04
7	6.19	4.96	99.14	5.06
8	6.22	4.96	99.17	5.07
9	6.20	4.95	99.18	5.08
10	6.18	4.93	99.38	5.08
Mean	6.21	4.96	99.29	5.07
R.S.D. (%)	0.2	0.2	0.2	0.3
(B) Method precision results in terms of mean recoveries (n = 2) in mg of each component per 5 ml				
1	6.22	4.94	99.17	5.07
2	6.20	4.94	99.18	5.08
3	6.21	4.94	99.93	5.10
4	6.23	5.00	99.55	5.08
5	6.25	4.98	99.16	5.07
6	6.25	4.99	99.21	5.06
Mean	6.23	4.97	99.37	5.08
R.S.D. (%)	0.3	0.6	0.3	0.3
(C) Robustness test results, precision of replicate sample analysis by second analyst, means of n = 2				
1	6.23	4.98	100.78	5.15
2	6.22	4.97	98.99	5.05
3	6.21	4.96	99.21	5.08
4	6.25	5.01	99.08	5.04
5	6.27	5.01	99.77	5.09
6	6.22	4.97	99.57	4.96
Mean	6.23	4.98	99.40	5.08
R.S.D. (%)	0.4	0.4	0.8	1.3

components between the two sets of six replicates (Table IIIB *vs.* Table IIIC) were no more than 0.2%, indicating ease of method transfer between labs.

System suitability

The chromatographic systems met the system suitability criteria of precision, resolution and tailing factors as described in the Experimental section indicating that they were performing satisfactorily and reproducibly.

The above findings on method selectivity, accuracy, precision and ruggedness, fulfill the requirements for validation of a stability-indicating HPLC method for regulatory submission. Improvements incorporated in these methods over previous methods were made in the areas of sample preparation, instrumentation parameters and actual chromatography. Sampling viscous syrups with a TC pipet employing the usual rinsing procedure was found to aid in method precision and accuracy as did dilution to the 0.2 mg/ml level for guaifenesin, 0.0125 mg/ml for PPA and 0.01 mg/ml for both DM and B. These changes made possible use of ordinary 20–25-μl loop autoinjectors with increased efficiency. Detector wavelength optimization at 273 nm for the B–G assay and 263 nm for the PPA–DM assay was also useful in this regard. Common mixed standards with dilution from the PPA–DM level to the B–G assay levels combined with a similar sample dilution for each assay provided an en-

hanced efficiency and time savings. Use of the column heater at 35–40°C gave reproducible retention times and peaks of decreased tailing factor.

CONCLUSIONS

The developed methods for phenylpropanolamine–dextromethorphan and for benzoate–guaifenesin make possible the rapid, accurate and precise measurement of these ingredients in the cough-cold product under investigation. They will provide sufficient quality data to support product stability claims as required for marketing a safe and effective composition.

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