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High-performance liquid chromatographic analysis of dextromethorphan, guaifenesin and benzoate in a cough syrup for stability testing[☆]

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Abstract

A method has been developed for the analysis of a cough syrup containing dextromethorphan, guaifenesin, benzoic acid, saccharin and other components. Forced degradation was also studied to demonstrate that the method could be employed during a stability study of the syrup. Final conditions were phosphate buffer (25 mM, pH 2.8) with triethylamine (TEA)–acetonitrile (75:25, v/v). In such conditions, all the actives, excipients and degradation products were baseline resolved in less than 14 min, and different wavelengths were used for the different analytes and related compounds.

Keywords: Analysis; Cough syrup; Validation; Forced degradation

1. Introduction

The commonly used cough syrup ingredients are a cough suppressant mean, an expectorant, a preservative and as part of the excipients sweeteners, acidulants and natural or artificial colouring and flavouring agents. These compounds are contained in the pharmaceutical form in very different proportions and present chemical forms of very different nature. All these aspects increase the analytical problem.

Previous GC and HPLC, and even CE methods have measured these constituents either individually or in different combinations. GC method included a syrup containing dextromethorphan, guaifenesin and diphenhydramine [1]. CE method determined dextromethorphan, diphenhydramine and phenylephrine [2]. LC methods have been applied for dextromethorphan [3]; triprolidine, pseudoephedrine, acetaminophen and dextromethorphan [4]; theophylline, guaifenesin and benzoic acid [5]; dextromethorphan, guaifenesin and benzoate [6]; acetaminophen, guaifenesin and dextromethorphan [7] and phenylpropanolamina, dextromethorphan, guaifenesin and benzoate in two separate methods [8]. The only method including the three actives [6] in one run employed an ion-pairing mobile phase with methanesulfonate and formic acid. The addition of ion-pair reagents to the mobile phase often results in methods that lack reproducibility and that pose a special problem with impurity profiling through a stability test. Moreover, at 230 nm, the wavelength necessary to obtain enough sensitivity for dextromethorphan impurities, the baseline was noisy.

The objective of the present work was the development and validation of a method for a cough syrup containing dextromethorphan, the cough suppressant active with an amine group, guaifenesin, the expectorant with alcohol and ether groups, benzoic acid, the preservative with acidic nature and saccharin as sweetener, among other components, such as citric acid and strawberry flavour. The individual compounds

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and the pharmaceutical form underwent forced degradation to obtain the impurities profile and to demonstrate that the method could be employed during a stability study of the syrup.

2. Experimental

2.1. Apparatus

An Agilent technologies 1100 series (Las Rozas, Madrid, España) provided with an automatic injector, a diode-array detector and a column oven. The chromatographic analysis was performed on a 5 μm particle, Discovery C_{18} column (Supelco, Alcobendas, Madrid, Spain), 25 cm × 0.46 cm, kept at 35 °C.

Final chromatographic conditions were an isocratic elution with phosphate buffer (25 mM, pH 2.8)–acetonitrile (75:25, v/v). The phosphate buffer was prepared from H_3PO_4 by adding triethylamine (TEA) to reach the pH 2.8. The flow rate was 1 ml/min and the injection volume was 20 μl. UV detection was performed at different wavelengths: 290 nm for the identification of saccharin and the quantification of dextromethorphan and benzoate, and 250 nm for the quantification of guaifenesin. For impurities: 290 nm for guaifenesin and benzoate related compounds, and 230 nm for dextromethorphan because a higher sensitivity was needed for the degradation products of this analyte.

2.2. Chemicals

Standards of individual compounds, as well as the syrup, and excipients of the speciality were kindly provided by Cinfa (Pamplona, Spain). NaOH and TEA came from Panreac (Barcelona, Spain) and H_3PO_4 and acetonitrile from Merck (Darmstadt, Germany).

2.3. Standard solutions and sample preparation for quantification

A stock solution of the analytes was prepared with 50.0 mg of dextromethorphan hydrobromide, 500.0 mg of guaifenesin, 87.5 mg of sodium benzoate and 62.5 mg of saccharin exactly weighed and dissolved in a 25 ml volumetric flask with purified water. For the working standard, 2.5 ml of the stock solution was made up to 25 ml with mobile phase.

For samples, 2.5 ml of sample was dissolved in a 25 ml volumetric flask. An aliquot was filtered with a 0.45 μm syringe filtration disk to the vials for injection in the HPLC system.

2.4. Validation

Validation was developed following ICH guidelines. *The selectivity* was tested by running solutions containing the placebo of the speciality in the same quantities and conditions as the samples, to show that there was no peak in the retention times corresponding to the analytes. Moreover, solutions submitted to forced degradation were also run to show both the resolution and selectivity of the method. In all cases, the spectra of the peaks were compared with those of the pure standards.

For the linearity assay, guaifenesin concentrations ranged from 1.5 to 2.4 mg/ml, for benzoate from 0.26 to 0.42 mg/ml and for dextromethorphan hydrobromide from 0.15 to 0.24 mg/ml. They were prepared by adding 750 μl (75%), 900 μl (90%), 1000 μl (100%), 1.1 ml (110%) and 1.2 ml (120%) of stock solution in 10 ml volumetric flasks and completed the total volume with mobile phase. Each point was analysed three times.

Accuracy was tested by triplicate at five levels (from 75 to 120%), and in parallel with the linearity assay for the three main components. Samples were prepared in the same way but including the corresponding excipients. The percentage recovery and RSD were then calculated.

Instrumental precision was tested by running consecutively 10 injections of the same standard. Then, repeatability or *intra*-assay precision was tested. *Intra*-assay precision data were obtained by repeatedly analyzing, in one laboratory on 1 day, nine aliquots of a homogeneous sample, each of which was independently prepared according to the method procedure. The second type is intermediate precision. These data were obtained by repeating the intra-assay experiment on a different day with newly prepared mobile phase and samples.

The same parameters were evaluated not only for the quantification of main component range, but also for the impurities ranging from 0.05 to 1%.

The quantitation limit (LOQ) is the lowest level of analyte that can be accurately and precisely measured. LOQs were calculated following EURACHEM recommendations [9] by testing the repeatability of successive injections of standard solutions at the low concentration levels. Based on the RSD obtained, 10% was considered acceptable for impurities at the LOQ. Limits of detection were estimated with the signal/noise ratio of 3.

2.5. Forced degradation

Forced degradation was conducted to demonstrate that the method was stability indicating. Separate solutions were prepared containing each substance, the excipients and the drug product at the working standard concentration: 2 mg/ml for guaifenesin, 0.35 mg/ml for benzoate, 0.25 mg/ml for saccharin and 0.2 mg/ml for dextrometorphan. They were exposed to the following conditions: (1) acid degradation with a final concentration of 0.1 M HCl; (2) base degradation with 0.1 M NaOH; (3) hydrogen peroxide degradation with 3% (v/v) of final peroxide concentration in the flask; (4) heat in an oven at 90 °C for 1 h; (5) light with UV lamp at 254 nm.

Solutions were analysed by HPLC at the start and after 24 h of degradation. Samples exposed to base degradation were neutralised prior to their measurement. The initial peak area

Table 1
Tailing factor for saccharin, guafenesin, benzoate and dextrometorphan

	Guaifenesin			Benzoate			Dextromethorphan		
	t_R	Tailing factor	Symmetry	t_R	Tailing factor	Symmetry	t_R	Tailing factor	Symmetry
Atlantis dC18 (Agilent)	7.477	1.072	0.927	14.812	1.086	0.889	21.409	6.944	0.102
Zorbax SB (Agilent)	5.462	0.956	1.081	10.719	1.238	0.767	13.848	4.387	0.14
Purospher Star RP_{18} (Merk)	6.914	1.009	1.000	13.782	1.096	0.873	18.53	3.599	0.212
Shymmetry Shield RP_8 (Waters)	5.874	0.999	0.983	14.783	1.023	0.939	6.639	2.233	0.341
Shymmetry Shield RP_{18} (Waters)	5.758	0.959	1.059	14.99	1.06	0.906	6.126	1.879	0.415
Discovery C_{18} (Supelco)	5.592	1.097	0.908	9.52	1.277	0.732	12.854	2.894	0.254

was considered 100% to establish the corresponding mass balance after degradation.

3. Results and discussion

During development, the main problem was dextromethorphan tailing, which was not avoided with classical approaches such as triethylamine addition to the mobile phase.

New generations of stationary phases are being developed with better characteristics. Trace metals in the silica increase silanol acidity and peak asymmetry. These silanol interactions are reduced or eliminated by choosing a less acidic, ultra-pure silica. Therefore, our efforts were first focused on testing different new HPLC columns, shown in Table 1, with some of their performance parameters. Best results considering dextromethorphan tailing factor corresponded to SymmetryShield both RP_{18} and RP_8, and Discovery C_{18}, all of them 250 mm × 4.6 mm and tested employing the same mobile phase.

The lower tailing factor obtained with the SymmetryShield columns was partly due to a different selectivity with lower retention of dextromethorphan and higher retention of benzoate than other columns. This made it difficult to separate dextromethorphan from guaifenesin and even saccharin with a short total run time. Therefore, the method was finally developed with the Discovery C_{18} column.

Final conditions were phosphate buffer (25 mM, pH 2.8) with TEA–acetonitrile (75:25, v/v). In such conditions, all the actives, excipients and degradation products were baseline resolved in less than 14 min, as can be observed in Figs. 1–3, for each wavelength. Saccharin was clearly identified and it could be quantified as pre-validation assays showed, but it was not included in the formal validation assay because it is considered an excipient and its quantification was not necessary.

3.1. Validation

Both standards and samples showed a good linearity for the three analytes in the two ranges with correlation coefficients over 0.999, except for benzoate in the low range, which was over 0.99. The regression equations are shown

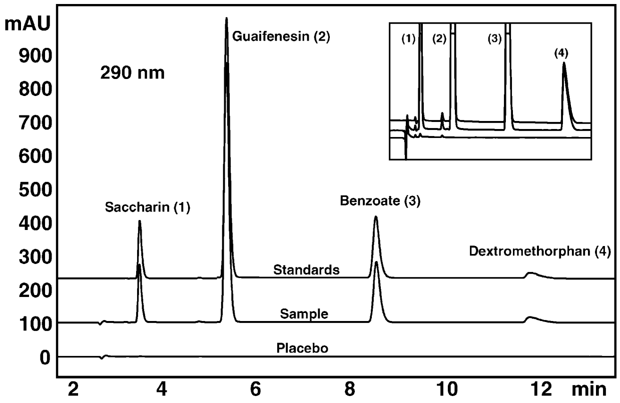

Fig. 1. Chromatogram of excipients, sample and standards at 290 nm.

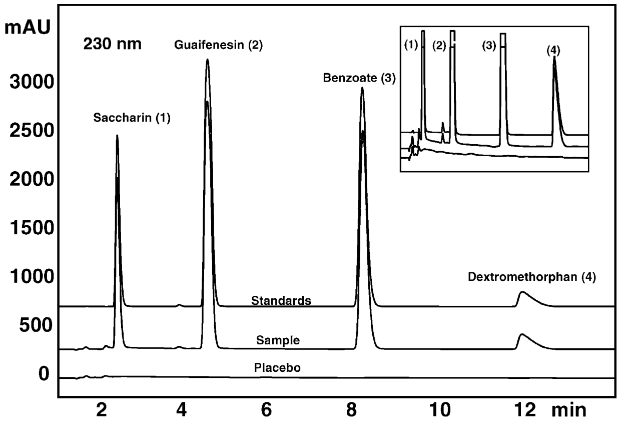

Fig. 2. Chromatogram of excipients, sample and standards at 230 nm.

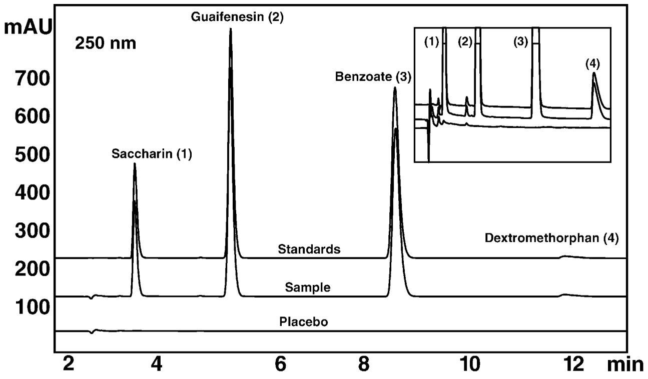

Fig. 3. Chromatogram of excipients, sample and standards at 250 nm.

Table 2
Linearity parameters for guaifenesin, benzoate and dextromethorphan

Linearity		Guaifenesin	Benzoate	Dextromethorphan
Standards	Range (mg/ml)	1.501–2.401	0.263–0.421	0.150–0.241
Quantification range	Slope ± C.L.	2167.64 ± 78.12	4791.05 ± 152.96	1608.30 ± 49.85
	Intercept ± C.L.	175.35 ± 120.23	75.76 ± 53.79	2.08 ± 10.02
	r	0.999	0.999	0.999
Samples	Range (mg/ml)	0.001–0.0200	0.0002–0.0035	0.0001–0.0020
Impurities range	Slope ± C.L.	3426.40 ± 15.35	5203.46 ± 125.63	17214093 ± 224.86
	Intercept ± C.L.	1.76 ± 0.18	1.29 ± 0.26	1.84 ± 0.27
	r	1.000	0.998	0.999
	L.D. (%)	0.010	0.120	0.040
	L.C. (%)	0.004	0.037	0.010

in Table 2. A small bias was found in some of the regression lines, because the intercepts with their limits of confidence did not include the zero value. It could be mostly justified by the good fit of the points to the regression lines, which makes the limits of confidence for the intercept very narrow; they posed no practical effect. Curiously, guaifenesin peak, in spite of being a narrow and symmetrical peak and with absorbance under 0.8 AU, resulted not linear at 290 nm with a response factor decreasing with an increasing concentration of the analyte. This was why a valley in the UV spectra was chosen for the measurement with good results.

RSD values in the *intra*-assay instrumental precision ranged from 0.09 to 0.23% for the three analytes in the upper level, and from 0.32 to 5.49% in the impurities level. For intermediate instrumental precision, RSD ranged from 0.31 to 0.35% in the main components level, and from 0.40 to 3.94% in the lower level. For the *intra*-assay precision of the method, RSDs ranged from 0.26 to 1.31%, including both standards and samples. For intermediate precision of the method, RSDs ranged from 0.89 to 0.95%. As could be expected, dextromethorphan, the compound with lower signal, presented the higher variability.

Recoveries do not statistically differ from 100% (t-test, $P < 0.05$) in the upper level with RSD ranging from 0.56 to 0.85%. In the lower level, recovery differed from 100% only for dextromethorphan in samples, which was 106%. This is probably due to some small peak from the excipients co-eluting at this time, but it was not considered of any importance because dextromethorphan will never be measured at this level. The assay was designed to show the possibility of measuring its impurities at that level. Moreover, the acceptance criteria would be 90–110%. Limits of quantification were under 0.05% for the three analytes.

3.2. *Forced degradation*

Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods. Forced degradation studies should be performed prior to implementation of stability studies to assure analytical methods are stability indicating. The only analyte showing a measurable degradation in the tested conditions was dextrometorphan, which was degraded in a 13% with oxidant media. The peaks obtained were well separated and fulfilled the mass balance in good approach.

4. Conclusions

A method has been developed for the analysis of a cough syrup containing dextromethorphan, guaifenesin, benzoic acid and saccharin, among other components, such as citric acid and strawberry flavouring. Forced degradation was also studied to demonstrate that the method could be employed during a stability study of the syrup.

The method was validated following ICH guidelines for selectivity, linearity, precision and accuracy, and all the parameters permitted us to consider the method reliable. Limits of detection and quantification were also obtained and they showed that impurities could be detected under the 0.1% level.

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