KNAUER

Application Note

► Ingredients in cough syrup

Category	Pharmaceutical
Matrix	Cough syrup
Method	HPLC
Keywords	Cough syrup, guaifenesin, codeine, pseudoephedrine
Analytes	guaifenesin, codeine, pseudoephedrine
ID	**VPH1**, first publ. 12/07, updated 09/09

Summary

A fast and robust HPLC method for the simultaneous determination of ingredients in cough syrup is presented. The separation of codeine, pseudoephedrine and guaifenesin by means of reversed-phase HPLC has been found optimal.

Introduction

By order of a pharmaceutic company it should be devised a qualified method for the isocratic determination of codeine, pseudoephedrine and guaifenesin in cough syrup. The intention was to get a definite separation of all ingredients, to apply this method for quality control. The additional separation of an additive, which is only known to the manufacturer and has not to be determined, essentially affects the choice of stationary phases. The mentioned ingredients play different roles for the therapy of bronchial diseases. Guaifenesin has a mucolytic function and at the same time it suppresses dry cough. Codeine is the monomethylether of morphine and is found in opium. It is classified as narcotic and affects not only depressant on cough but also controvert pain generally. Usually codeine is used in combination with other pharmaceuticals. Pseudoephedrine acts against allergies and has a decongesting effect on the mucous membrane. Wilcox and Stewart [1] describe a method for determination of guaifenesin. For the separation of guaifenesin and codeine they use underivatized silica gel and apply phosphate buffer and acetonitrile. But this method has the problem, that pseudoephedrine can not be separated from codeine sufficiently. Other literature [2] describes the separation of guaifenesin and pseudoephedrine by supercritical fluid chromatography. The isocratic separation of acetaminophen, pseudoephedrine, guaifenesin and dextromethorphan is described by the use of a phenyl phase and by means of a ternary mobile phase. This seems to be more extensive in comparison to the isocratic HPLC method and therefore we look about the pharmaceutic guide for analytics, which proposes a reversed-phase separation for guaifenesin [3]. Based on molecule structure (Fig. 1) different reversed-phase C18 silica gels should be qualified for the separation of the named compounds. Because of the fourth ingredient in the cough syrup, unknown by name and structure, C18 reversed-phase materials with different polar selectivity were chosen for method-development. To obtain symmetric peaks for all substances, the pH was adjusted to 1.85. On the basis of adequate pH-stability, three different base deactivated C18 phases with different C18 density and polarity were applied. The following stationary phases were used: ProntoSIL 120-5 C18 ace-EPS, Eurospher 100-5 C18 and ProntoSIL 120-5 C18 SH.

Experimental
sample preparation

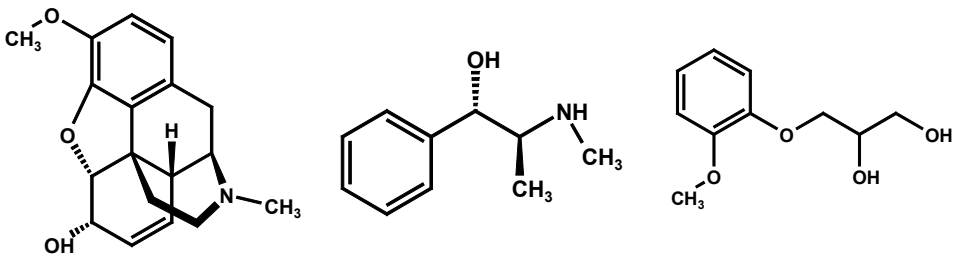
The sample preparation is very simple and only requires a dilution step. For the analysis of the cough syrup, the original sample was diluted 1:25 with HPLC eluent. It is recommendable to filter the sample with a 0.45 µm syringe filter unit.

Experimental preparation of standard solution

For all three standards a calibration curve in the range of 50 to 1000 mg/l was generated by means of a 5-point calibration with the following regression equations:

codeine	y=0.020x+0.059	R^2=0.997
pseudoephedrine	y=0.010x+0.143	R^2=0.999
guaifenesin	y=0.011x+0.195	R^2=0.999

Chemical structures



codeine pseudoephedrine guaifenesin

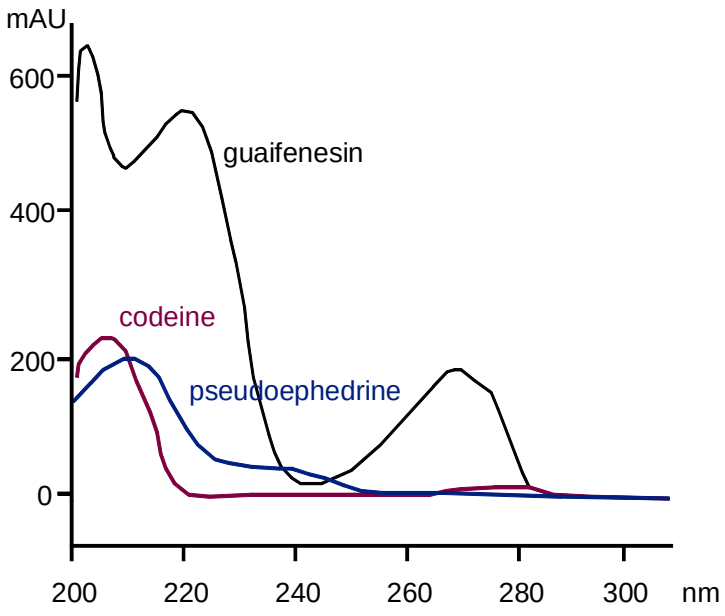


Fig. 1

Wavelength spectrum of codeine, pseudoephedrine, and guaifenesin

Method parameters

Column	ProntoSIL 120-5 C18 ace-EPS 250 x 4 mm Eurospher 100-5 C18 250 x 4 mm ProntoSIL 120-5-C18 SH 250 x 4 mm
Eluent	H_2O:MeOH (70:30, v/v) with 0.1% TFA (pH 1.85)
Flow rate	0.8 ml/min
Injection volume	5 µl
Column temperature	40 °C
Detection	UV
	codeine / pseudoephedrine λ_{max} 210nm
	guaifenesin λ_{max} 220nm

Results

With all applied stationary phases an adequate separation of the standard-mixture could be obtained. The elution order of the investigated compounds with the listed stationary phases shows no differences. Only the selectivity of the polar modified phase results in a lower α-value for the separation of codeine and pseudoephedrine. The analyses of the samples demonstrate that only with the ProntoSIL C18 SH column, a good separation of the additive from the other ingredients could be achieved (Fig. 2 blue trace). This way a reliable quantification for the analyzed substances can be realized in presence of the unknown ingredient (Table 1).

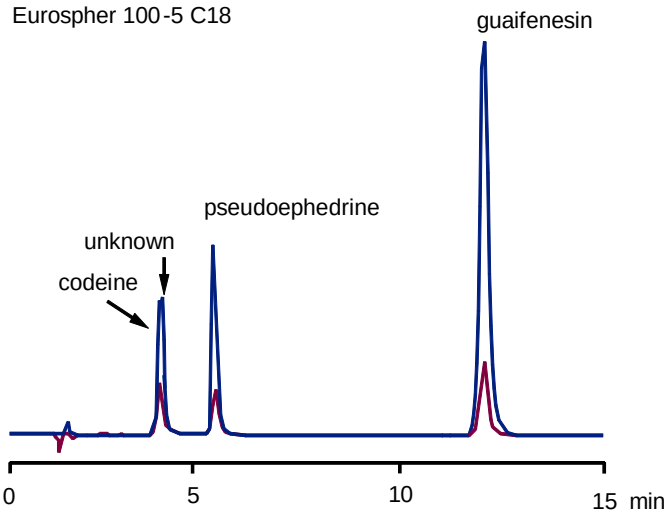


Fig. 1

Overlays of standard (red) and diluted sample (blue) on the Eurospher 100-5 C18 phase

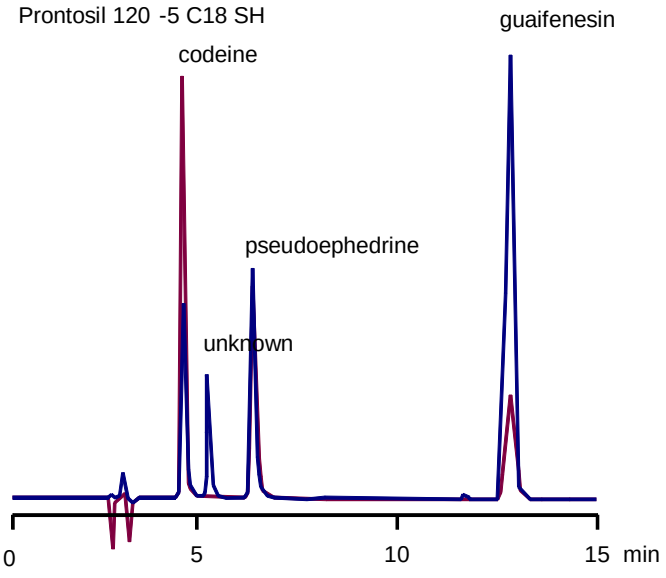


Fig. 2

Overlays of standard (red) and diluted sample (blue) on the ProntoSil 120-5 C18 SH phase

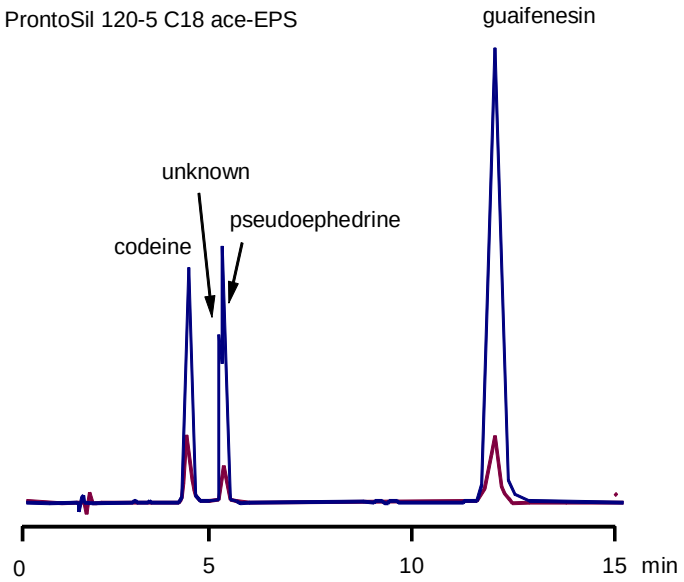


Fig. 3

Overlays of standard (red) and diluted sample (blue) on the ProntoSil 120-5 C18 ace-EPS phase

Table 1

Results for quantitative determination of ingredients in cough syrup

Substance	Concentration [g/l]	Concentration in 5 ml [mg]	Manufacturer's data [mg/5 ml]
codeine	2.048	10.24	10
pseudoephedrine	6.095	30.48	30
guaifenesin	20.65	103.28	100

Method performance

Linearity (r^2)	0.997-0.999
Linearity range	50 to 1000 mg
Retention time precision	< 1 % RSD
Peak area precision	< 3 % RSD

Conclusion

The stationary phase with the highest carbon load and an increased steric selectivity, obtains the best separation results including a definite separation of the additive from the named ingredients. Both, the polar modified C18 phase with integrated polar groups, and the standard C18 phase could not attain a complete separation of the additive and codeine or pseudoephedrine. This is an excellent example to demonstrate the importance of considering the real sample including by-products for HPLC method development.

References

[1] M.L. Wilcox, J.T. Stewart, Journal of Pharmaceutical and Biomedical Analysis, 2000 Vol. 23, Nr. 5, S. 909-916

[2] K.M. Patil, I. Choir, B. Raman, M. Sundaresan, Indian Journal of Pharmaceutical Sciences, 2001 Vol. 63, Nr. 6, S. 468-472

[3] G. Lunn, N.R. Schmuff, HPLC Methods for Pharmaceutical Analysis, John Wiley & Sons 1997

Author

Silvia Marten, Head of Columns and Applications Department, KNAUER

Physical properties of recommended columns

The ProntoSIL C18 ace-EPS belongs to the new group of stationary RP supports with polar embedded groups. The packing is very stable over a wide pH range (pH 1-10). In comparison to a classical bonded C18 column acidic compounds show a higher retention whereas basic compounds show a slight decrease of retention on an embedded polar column.

Stationary phase	ProntoSIL 120-5 C18 ace-EPS
USP code	L1
Pore size	120 Å
Particle size	5 µm
Shape	spherical
Surface area	300 m^2/g
% C	18.5
Endcapping	yes
Dimensions	250 x 4 mm
Order number	25DF18APSJ

Eurospher C18 is a premium, silica-based HPLC packing material developed by Knauer. It can be universally used in different application areas in the analytical as well as preparative range.

Stationary phase	Eurospher 100-5 C18
USP code	L1
Pore size	100 Å
Particle size	5 µm
Shape	spherical
Surface area	350 m^2/g
% C	15
Endcapping	yes
Dimensions	250 x 4 mm
Order number	25DE181ESJ

ProntoSIL C18 SH is the stationary phase in the ProntoSIL line with the highest carbon load. It is fully endcapped. Due to the carbon load it shows an excellent shape, selectivity and stability even at pH 1.

Stationary phase	ProntoSIL 120-5 C18 SH
USP code	L1
Pore size	120 Å
Particle size	5 µm
Shape	spherical
Surface area	300 m^2/g
% C	17
Endcapping	yes
Dimensions	250 x 4 mm
Order number	25DF180PSJ

Recommended instrumentation

This application requires the isocratic Smartline HPLC system, equipped with degasser, autosampler, column oven, and multi-wavelength UV detector.

Description	Order No.
Smartline Pump 1000, incl. 10 ml pump head	A50303
Smartline Manager 5000 with degasser	A5316
Autosampler 3950	A5005-1
Smartline Column Oven 4050	A5300
Smartline UV Detector 2600 PDA	A5200
10 mm Flow Cell	A4061
ChromGate Software	A1493
ChromGate PDA License for Detector 2600	A1459

Contact information

Wissenschaftliche Gerätebau
Dr. Ing. Herbert Knauer GmbH
Hegauer Weg 38
14163 Berlin, Germany

Tel: +49 (0)30 / 809727-0
Fax: +49 (0)30 / 8015010
Email: info@knauer.net
Internet: www.knauer.net