

HPLC-UV Determination of Dextromethorphan in Syrup

Method validation

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A HPLC-UV method, for determination of dextromethorphan hydrobromide in syrup, was validated. The chromatographic analysis was performed using an RP-18, Nucleodur chromatographic column (250 mm × 4 mm, 5 µm) at constant temperature (50°C) with a mobile phase consisting of a mixture of acetonitrile/methanol (70:30 v/v) with sodium docusate (as ion pair agent) and ammonium nitrate, pH = 3.4. The flow rate of the mobile phase was 1 mL/min and the detection was carried out at 280 nm. System suitability, specificity, linearity, precision, accuracy, limit of detection and limit of quantification agreed with current pharmacopeial requests. The method is suitable for routine analysis of dextromethorphan hydrobromide in syrup.

Keywords: dextromethorphan, HPLC, validation, syrup

Dextromethorphan hydrobromide (DXHB) has been used as antitussive for more than half a century [1], single or in combinations [2]. DXHB is a non-selective serotonin uptake inhibitor and an agonist of sigma-1 receptor, and acts centrally to elevate threshold for coughing [3,4]. The activity of DXHB on N-methyl-D-aspartate receptor might have a contribution on antitussive effect, also for cough in palliative care [5,6].

Many papers describing DXHB analysis in pharmaceuticals (single or in combinations) and biological matrices, have been published: LC-MS [7], GC-MS [8,9], cyclic voltammetry [10], capillary electrophoresis [11, 12], potentiometric [13, 14], spectrophotometric [14-17], LC-UV [18-25]. This paper describes the development and validation of an HPLC-UV method for the determination of DXHB in syrup, considering latest validation guidelines and protocols [26-33].

Experimental part

Materials and methods

Standards, reagents and pharmaceutical substances

Glycerol (batch 1555415) and pharmaceutical sugar (batch 7161T43034) were supplied by AAK Sweden AB, Sweden and Tereos, France, respectively. DXBH, batch 4, was supplied by EDQM.

All reagents were analytical or HPLC grade: dioctyl sulfosuccinate sodium salt, batch A0258923 (Acros Organics, Belgium), glacial acetic acid, batch IL020161143J (International Laboratory, USA), ammonium nitrate, batch 060442300 (Chimopar, Romania), acetonitrile, batch 01507820 (Titolchimica SRL, Italy). All experiments were performed using ultrapure water.

Apparatus and chromatographic conditions

Ultrapure water was prepared by means of a Simplicity apparatus (Millipore), weighing was performed on a Mettler Toledo analytical balance, density (ρ_s) was determined by

means of KEM digital densimeter, chromatographic analysis was performed on an Rigol L-3000, HPLC system (quaternary pump, autosampler, column oven, DAD detector, Clarity chromatographic software). Samples were injected into an RP-18, Nucleodur chromatographic column (250 mm × 4 mm, 5 µm) at constant temperature (50°C). The mobile phase was prepared by solving 3.11 g of sodium docusate in a mixture consisting of 700 mL of acetonitrile and 300 mL of purified water, followed by the addition of 0.56 g of ammonium nitrate. The apparent pH was adjusted to 3.4 with glacial acetic acid. Injection volume was 20 µL, flowrate was 1 mL/min., detection wavelength was 280 nm.

Results and discussions

Synthetic mixtures of the drug product components, preparation and sample preparation

In order to perform the validation of the method several synthetic mixtures of the drug product components (SMDPC), according to Annex 4 of the Marketing Authorization (MA) for the commercial product Tussin 6.5 mg/ 5 mL, syrup, considering different concentration levels of DXBH (0% - 80%-90%-100%-110%-120%) as stated in table 1.

An appropriate amount (≈ 10 g) of SMDPC, all series, except serie 7, was weighed in 100 mL volumetric flasks. All flasks were filled to mark with purified water and sonicated for 5 minutes and a filtration through acrodisc filters was performed prior to injection. For all series, except serie 7, the relative density was determined.

Method validation

HPLC-UV method was validated in terms of system suitability, specificity, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ).

System suitability was assessed considering the following acceptance criteria: peak repeatability (area RSD

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Table 1
SYNTHETIC MIXTURES OF THE DRUG PRODUCT COMPONENTS

SMDPC Volume (mL)	50 mL									
Concentration level	0%	80%	90%	100%	110%	120%	100%	100%	100%	Standard
Number of Serie	1	2	3	4	5	6	8	9	10	7
DXHB (mg)	0	52.13	58.48	65.21	71.48	78.17	64.11	64.07	65.04	5.05
Sugar (mg)	According to Annex 4 of MA for Tussin 6.5 mg/5 mL									0
Glycerol (mg)	According to Annex 4 of MA for Tussin 6.5 mg/5 mL									0
Purified water (up to)	50 mL									

Serie	2	3	4	5	6
F _{calculated}	0.5154	0.2894	0.5154	0.4228	1.7366
F _{theoretical}	6.3882				
t _{calculated}	1.5368	1.4720	1.3837	1.2225	1.7289
t _{theoretical}	2.3060				

Table 2
STATISTICAL DATA FOR SPECIFICITY

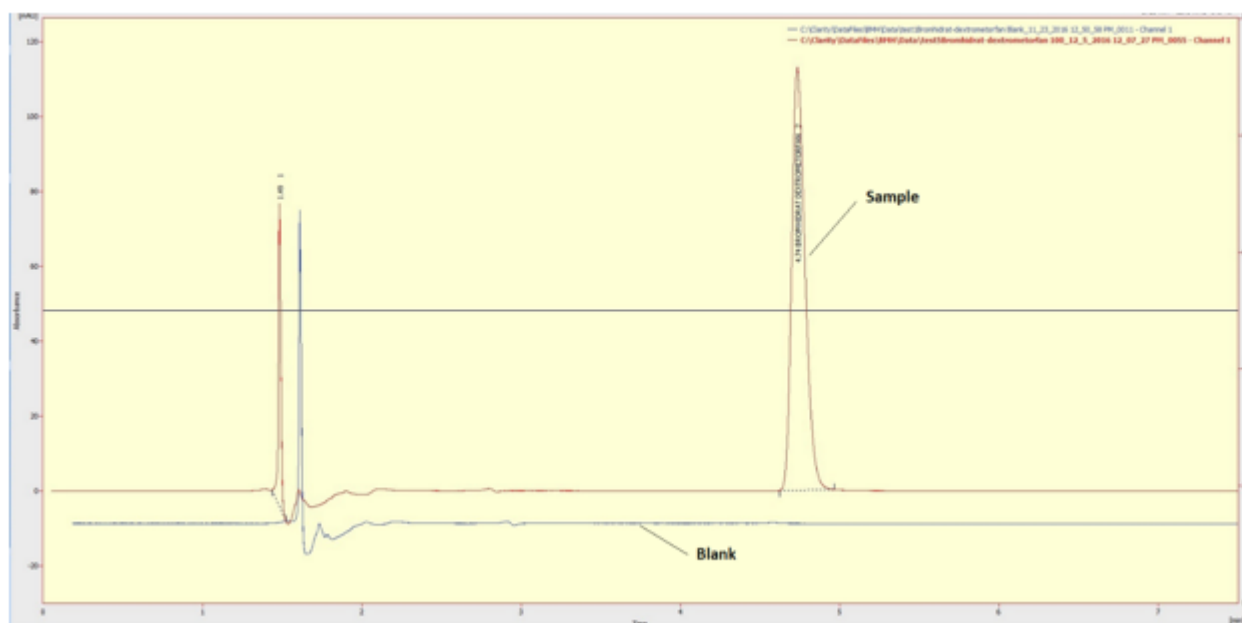


Fig. 1. Overlaid chromatograms of samples and blank

for 5 injections, serie 7) < 0.73%, asymmetry 0.8-1.6, column efficacy (theoretical plates -N) > 2000. Area RSD for 5 injections was 0.615%, asymmetry ranged 1.422 and 1.439 and N > 15480. All system suitability criteria were fulfilled.

Specificity was verified by performing 7 determinations (5 injection/determination) for series 1, 2, 3, 4, 5, 6 and 7. DXHB retention times of series 2, 3, 4, 5, and 6 were statistically compared with those related to serie 7, by means of t-Student and Cochran tests. Result are presented in table 2. Overlaid chromatograms of series 1, 2, 3, 4, 5 and 6 are showed in figure 1. Retention time for DXHB was about 4.7 min.

The information presented in table 2 and figure 1, leads us to the conclusion that serie 1 showed no analytical signal, $F_{calculated} < F_{theoretical}$ (the results are reproducible), $t_{calculated} < t_{theoretical}$ (the means do not differ, statistically speaking), so developed method is specific.

Linearity was tested at 5 concentration levels (series 2, 3, 4, 5 and 6), 5 replicates for each level. Acceptance criteria is correlation coefficient (r) > 0.99. The results, related to linearity, are presented in table 3.

The calibration curve parameters were calculated in Microsoft Excel, and the calibration curve is presented in equation (1) and r = 0.9997.

$$A = 7169.7 \times C_s - 43.046 \quad (1)$$

The method proved to be linear in the range 0.0776-0.1163 mg/mL.

Precision of the method was proved by evaluating repeatability and reproducibility in three different days considering 100% concentration level (Series 8, 9 and 10), 5 replicates each day. Statistical analysis of precision consisted off: variances homogeneity evaluation (Cochran test), repeatability variation coefficient (CV%), and reproducibility variation coefficient (CV_r%), considering a 5% error probability and 15 (5 × 3) samples.

Table 3
LINEARITY RESULTS

Sample	Sample mass (g)	Sample density (g/mL) – ρ_s	Concentration of injected solution (mg/mL) – C_s	Theoretical concentration in SMDPC (mg/5 mL)	Mean area - A	Determined concentration in SMDPC (mg/5 mL)	Recovery (%)
Serie 2	10.0187	1.346	0.0776	5.213	518.517	5.2625	100.9501
Serie 3	10.0128	1.346	0.0870	5.848	583.073	5.8723	100.4156
Serie 4	10.0346	1.346	0.0972	6.521	640.628	6.3991	98.13116
Serie 5	10.0196	1.347	0.1063	7.148	715.799	7.1181	99.5811
Serie 6	10.0230	1.347	0.1163	7.817	798.8	7.8952	100.9999

Variances homogeneity (Cochrane test)	Repeatability variation coefficient (CV _r %)	Reproducibility variation coefficient (CV _R %)
Calculated values		
0.448	0.233	1.221
Theoretical values		
0.707	-	-
Acceptance criteria		
$C_{\text{calculated}} < C_{\text{theoretical}}$	-	-

Table 4
STATISTICAL EVALUATION OF
PRECISION

Both variation coefficients should be below 2%. Statistical evaluation of precision is presented in table 4.

As stated in table 4, all variances are homogeneous (since $C_{\text{calculated}} < C_{\text{theoretical}}$), and both variation coefficients are below 2%. The method is precise.

Accuracy of the method was estimated by means of recovery using the same samples described in linearity testing (series 2, 3, 4, 5 and 6) in triplicate.

Statistical analysis of accuracy was performed by: Cochrane test (intragroup variance evaluation), Fisher test (mean recovery validity), t Student test (confidence interval for mean recovery). Statistical evaluation of accuracy is presented in table 5.

As shown in table 5, all variances are homogeneous ($C_{\text{calculated}} < C_{\text{theoretical}}$), mean recoveries are valid ($F_{\text{calculated}} < F_{\text{theoretical}}$) and confidence interval is very close to 100%. The method is accurate.

Intragrup variance homogeneity (Cochrane test)	Medium recovery validity (Fischer test)	Confidence interval of the mean recovery (%)
Calculated values		
0.396	0.036	99.499-100.784
Theoretical values		
0.68	3.48	-
Acceptance criteria		
$C_{\text{calc.}} < C_{\text{theor.}}$	$F_{\text{calc.}} < F_{\text{theor.}}$	-

Table 5
STATISTICAL EVALUATION OF ACCURACY

Calibration curve slope	7169.7	
Standard deviation of blank	0.0099	
	mg/mL	µg/mL
LOD	4.142×10^{-6}	4.142×10^{-3}
LOQ	1.38×10^{-5}	1.38×10^{-2}

Table 6
LOD AND LOQ

Limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-to-noise approach and are presented in table 6.

Conclusions

A HPLC-UV method, for determination of dextromethorphan hydrobromide in syrup, was validated in terms of system suitability, specificity, linearity, precision, accuracy, limit of detection and limit of quantification. All system suitability criteria were fulfilled. The method is: specific (results are reproducible and the means do not differ, statistically speaking), linear (over the concentration range 0.0776 - 0.1163 mg/mL, correlation coefficient $r = 0.9997$), precise (all variances are homogeneous and both repeatability and reproducibility coefficients are below 2%), accurate (variances are homogeneous, mean recoveries are valid, and confidence interval is very close to 100%). LOD and LOQ are 4.142×10^{-3} µg/mL and 1.38×10^{-2} µg/mL, respectively. The HPLC-UV is suitable for routine quantitative determination of dextromethorphan hydrobromide in syrup.

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