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RP-UPLC Stability Indicating Assay Method for Simultaneous Estimation of Dextromethorphan Hydrobromide and Chlorpheniramine Maleate in Tablet Dosage Form

A.J. Patel✉, N.K. Patel, A.J. Vyas, A.B. Patel and A.I. Patel

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ABSTRACT

RP-UPLC method was developed and validated for the determination of chlorpheniramine maleate and dextromethorphan hydrobromide in tablet dosage form. Reverse phase waters acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 µm) column using isocratic mobile phase of 0.5 mL 0.1% TFA (trifluroacetic acid) in H_2O:CH_3CN (70:30 %v/v). The flow rate was 0.2 mL/min and 252 nm wavelength use for detection on PDA detector. The retention time of chlorpheniramine maleate was 1.2 min and 2.2 min for dextromethorphan hydrobromide. Chlorpheniramine maleate and dextromethorphan hydrobromide was subjected to stress conditions including acidic, alkaline, oxidation, photolysis and thermal degradation. The method was validated as per ICH guideline with respect to samples to specificity, precision, accuracy, linearity and robustness.

KEYWORDS

RP-UPLC, Dextromethorphan hydrobromide, Chlorpheniramine maleate.

INTRODUCTION

Dextromethorphan hydrobromide is chemically 3-methoxy-17-methyl-9α,13α,4α-morphinan hydrobrormide anhydrous, is an *N*-methyl-D-aspartate receptor antagonist work as cough suppressant [1]. Chlorpheniramine maleate, chemically 2-pyridine propanamine, γ-(4-chloro-phenyl) *N*,*N*-dimethyl-,(*Z*)-2-butenedioate, is H1 antagonist used in allergic reactions hay fever, rhinitis, urticarial and asthma [2]. Chlorpheniramine maleate is an antiallergic, which benefits to diminish cough associated with allergies by inhibit the effects of a chemical messenger, histamine. Dextromethorphan hydrobromide is a cough suppressant, which relieves cough by decreasing the activity of cough centre in the brain [3]. Structure of dextromethorphan hydrobromide and chlorpheniramine maleate is given in Fig. 1.

UPLC is a separation technique based upon principle of liquid chromatography, which developed 2 µm particles for stationary phase. As compare to HPLC, these particles function at elevated mobile phase linear velocities to affect increase in resolution, sensitivity and speed of analysis [4-6]. A stability indicating assay method (SIAM) is a quantitative analytical method used to identify a decrease in the amount of the active

Author affiliations:

Department of Pharmaceutical Quality Assurance, B.K. Mody Government Pharmacy College, Polytechnic Campus, Near Aji Dam, Rajkot-360003, India

✉To whom correspondence to be addressed:

E-mail: asthapatel20797@gmail.com

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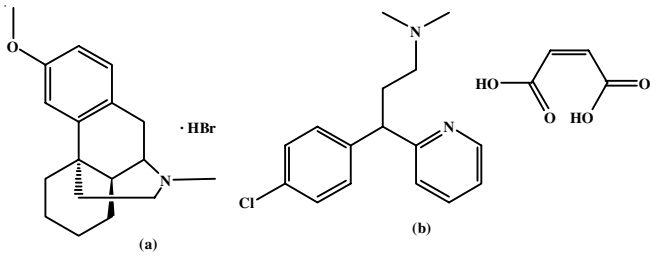

Fig. 1. Structure of (a) dextromethorphan HBr (b) chlorpheniramine maleate

pharmaceutical ingredient (API) existing due to the degradation. To provide evidence on how the quality of a drug substance or drug product differs with time under the effect of variety of environmental factor such as temperature, humidity, light, *etc*. Degradation of drug substances between 5-20 % has been accepted as reasonable for validation of chromatographic assay [7,8].

Review of literature for dextromethorphan hydrobromide and chlorpheniramine maleate shows that different analytical methods are available for estimation of combination drug. It includes HPLC [9-16], HPLC stability [17], UV [18], GC [19], UHPLC MS/MS [20], LC-MS/MS [21]. However, stability indicting HPLC method for syrup dosage form has not shown %degradation and degradation behaviour in oxidative and photolytic degradation condition only. So, it is worthwhile to develop stability indicating assay analytical method for dextromethorphan hydrobromide and chlorpheniramine maleate in tablet dosage form RP-UPLC. The develop method is applicable for routine analysis of dextromethorphan hydrobromide and chlorpheniramine maleate.

EXPERIMENTAL

Dextromethorphan·HBr (DXM) and chlorpheniramine maleate (CPM) samples were obtained from Contract Pharmacal Corporation (I) Pvt. Ltd., Ahmedabad, India. Coricidin·HBP tablets contains 30 mg dextromethorphan·HBr and 4 mg chlorpheniramine maleate. Solvents used were acetonitrile, trifluroacetic acid (TFA), water of HPLC grade.

Instrumentation and chromatographic conditions: Chromatographic separation was performed on reverse phase Waters Acquity UPLC BEH C18 (50 mm × 2.1 mm, 1.7 µm) column. The mobile phase consisted of 0.5 mL 0.1% TFA in H_2O:CH_3CN (70:30% v/v) with PDA detector at 252 nm. The flow rate was set at 0.2 mL/min. The injection volume was 5.0 µL; H_2O:CH_3CN (50:50% v/v) was used as a diluent while the column was maintained at 25 ºC.

Preparation of standard solution: Weighed 120 mg of dextromethorphan·HBr and 16 mg chlorpheniramine maleate into 100 mL volumetric flask and added about 50 mL of diluent and sonicated. Diluted the solution upto mark with diluent and mixed well. The solution formed will have concentration of dextromethorphan·HBr (DXM) and chlorpheniramine maleate (CPM) was 1200 µg/mL & 160 µg/mL , respectively. Transferred 5 mL of standard stock into 200 mL volumetric flask and diluted with diluent upto mark and mixed well (30 µg/mL DXM & 4 µg/mL CPM)

Assay of tablets: Coricidine·HBr (20 tablets) containing 4 mg CPM and 30 mg DXM were weighed and finely powdered. Blend equivalent to 4 tablets was placed in tablets into 100 mL volumetric flask. Added 60 mL of diluent, sonicated and diluted upto the mark with diluent and finally filtered (1200 µg/mL DXM & 160 µg/mL CPM). Withdrew 5 mL of solution into 200 mL with diluent and mixed well (30 µg/mL DXM & 4 µg/mL CPM).

Forced degradation study: Forced degradation studies of the drugs was performed under different stress conditions as mentioned in ICH guidelines Q1A (R_2) [22,23]. To perform forced degradation study samples were exposed to acidic, alkaline, oxidation, photolysis and thermal degradation. Acidic degradation was carried out using the 5 mL 1N HCl for 60 min at 60 ºC and the acidic solution was neutralized with base. Alkaline degradation was carried out using 5 mL 1N NaOH for 60 min at 60 ºC then the solution was neutralized with acid and diluted appropriately (30 µg/mL DXM & 4 µg/mL CPM) use CH_3CN:H_2O (50:50% v/v) as diluent then filter the both solution. Oxidative degradation was carried out using 5 mL 3% H_2O_2 for 2 h at room temperature. Photolytic degradation was carried out using UV Chamber for 1 week. Thermal degradation was carried out at 108 °C for 24 h. Diluted appropriately (30 µg/mL DXM & 4 µg/mL CPM) use CH_3CN:H_2O (50:50% v/v) as diluent and injected in UPLC system.

Method validation: The developed UPLC method was validated for parameters like system suitability, linearity, specificity, accuracy, precision, LOD, LOQ and robustness according to ICH guidelines Q_2 (R_1).

Linearity: The linearity of test solutions was prepared from dextromethorphan·HBr and chlorpheniramine maleate solution at five concentration levels from 50 to 150% of assay concentration and Linearity was carried out using concentration range of 2-6 µg/mL of CPM and 14-45 µg/mL of DXM.

Specificity: Specificity was performed by injecting diluent, placebo and sample solution to check the interference of excipients.

Accuracy: The accuracy was carried out in triplicate using three different concentration levels 50, 100 and 150% (2, 4 and 6 µg/mL for CPM and 14, 28 and 42 µg/mL for DXM). Study was performed by spiking above concentration to placebo. Accuracy method was performed by calculating percentage recovery.

Precision: Repeatability was performed under 6 replicates of chlorpheniramine maleate (4 µg/mL) and dextromethorphan HBr (30 µg/mL). Intra-day and inter-day variations of CPM and DXM were performed in triplicate at three different concentration levels 50, 100, 150% and result was expressed in term of RSD.

LOD and LOQ: The limit of detection (LOD) and limit of quantification (LOQ) were calculated by formula.

Robustness: Robustness of the method was verified by altering the chromatographic condition like change in wavelength ± 1 nm (251 and 253 nm), change in flow rate ± 0.02 mL/min (0.18 and 0.22 mL/min) and change in column temperature ± 2 ºC (23 and 27 ºC).

RESULTS AND DISCUSSION

Optimized UPLC conditions: The optimized mobile phase was 0.5 mL 0.1% TFA in H_2O:CH_3CN (70:30 %v/v). Chromatographic separation was performed using Waters Acquity

UPLC BEH C18 (50 mm × 2.1mm, 1.7 µm) column as stationary phase. The flow rate was set to be 0.2 mL/min. The injection volume was 2 µL and the detection was carried out at 252 nm. Optimized chromatograph is shown in Fig. 2. System suitability parameters are acceptable as resolution greater than 2. Theoretical plates should be > than 2000. Tailing factor is less than 2 and the system suitability parameters data is shown in Table-1.

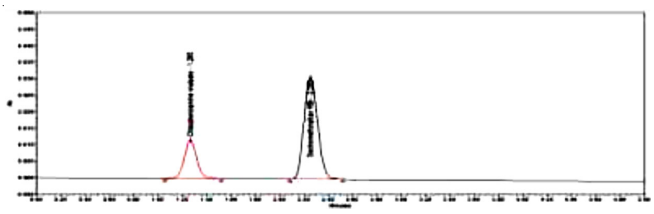

Fig. 2. Optimized chromatograph of chlorpheniramine maleate and dextromethorphan HBr

TABLE-1
OPTIMIZED CHROMATOGRAPHIC CONDITION

System suitability parameters	Chlorpheniramine maleate	Dextromethorphan HBr
Retention time	1.235	2.202
Theoretical plate	2743	3675
Resolution	–	4.61
Tailing factor	1.15	1.11

Forced degradation study: The main peak was well separated from degradant peak in alkaline degradation (Fig. 3). The degradation was within the range of 5-20 %. Oxidative degradation was more in chlorpheniramine maleate and photolytic degradation was more in dextromethorphan·HBr. In all the degradation condition peak purity angle was less than purity threshold so peak purity test pass (Table-2).

Method validation

Linearity: Linearity range for chlorpheniramine maleate and dextromethorphan·HBr was 2-6 µg/mL, R^2 = 0.9974 and 15-45 µg/mL R^2 = 0.9997, respectively. Chlorpheniramine maleate and dextromethorphan·HBr linearity graph is shown in Fig. 4.

LOD & LOQ: LOD and LOQ values were found to be 0.00711 and 0.02155 µg/mL for chlorpheniramine maleate and 0.00123 and 0.00374 µg/mL for dextromethorphan·HBr, respectively.

Precision and accuracy: Repeatability, intra-day and inter-day precision for RP-UPLC method was measured in terms of RSD found to be less than 2. Thus method is precise and accurate, since %recovery was found to be 99.1-100.6 for CPM and 98.8-100.8 for dextromethorphan·HBr (Table-3).

Robustness: Making a deliberate changes in wavelength, flow rate, temperature were take place and RSD of peak area found to be less than 2, specify that the method is robust and

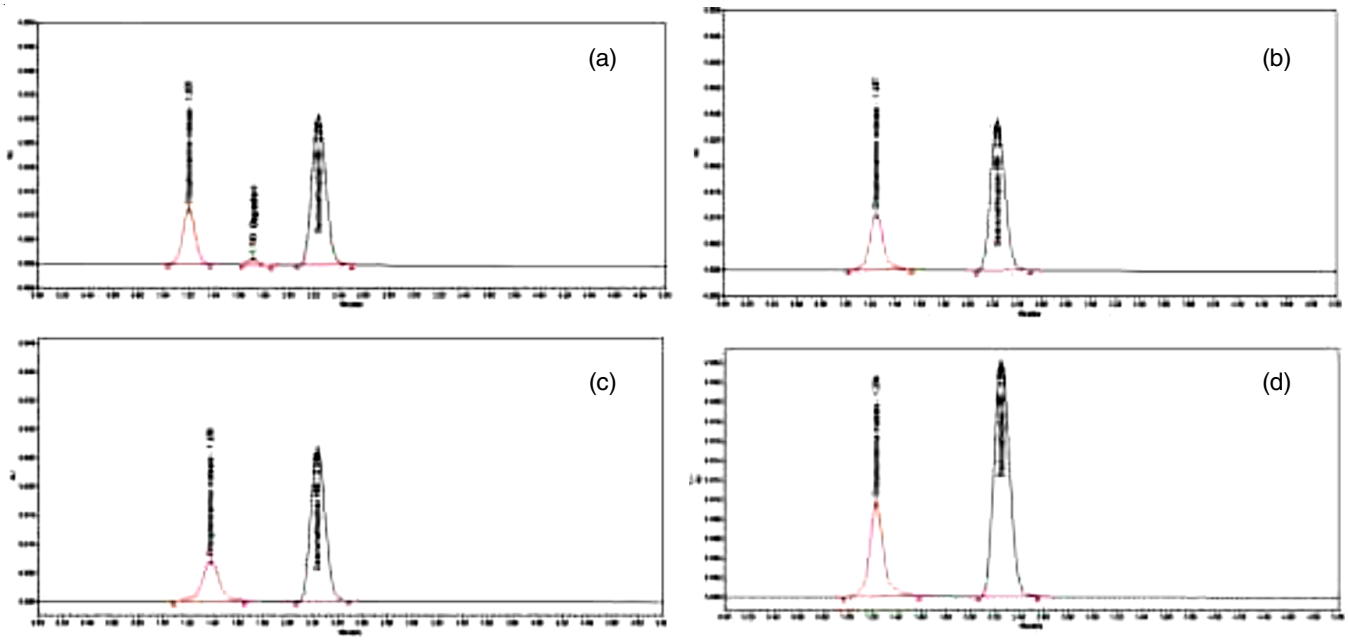

Fig. 3. Chromatogram of sample (a) under 1 N HCl_5 mL_60 °C_60 min, (b) under 1 N NaOH_5 mL_60°C_60 min, (c) under 3% H_2O_2_5 mL_RT_2 h, (d) under UV chamber for 1 week (e) under thermal degradation at 108 °C _ 24 h

TABLE-2
DEGRADATION CONDITION

Degradation method	Chlorpheniramine maleate (CPM)			Dextromethorphan HBr (DXM)		
	Degradation (%)	Purity angle	Purity threshold	Degradation (%)	Purity angle	Purity threshold
Acid degradation	6.6	0.345	3.478	8.2	0.382	15.873
Alkali degradation	10.4	0.942	3.541	11.6	0.233	47.030
Oxidative degradation	19.3	0.942	6.564	10.0	0.431	20.343
Photolytic degradation	6.4	0.891	0.946	14.4	0.435	0.946
Thermal degradation	8.7	0.477	0.969	9.1	0.474	17.420

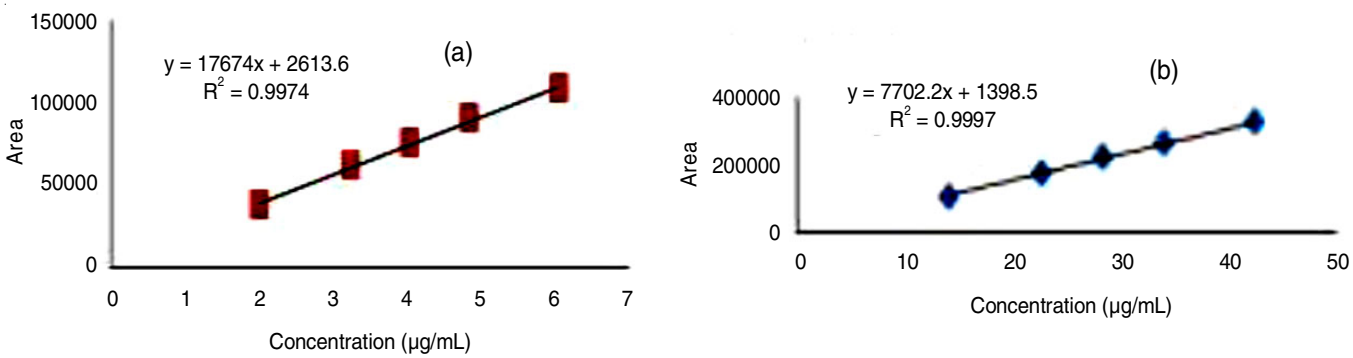

Fig. 4. Linearity graph of (a) chlorpheniramine maleate (b) dextromethorphan hydrobromide

TABLE-3
INTRA- AND INTER-DAY PRECISION AND ACCURACY OF METHOD

Drug name	Precision Level (%)	Conc. (µg/mL)	Intra-day precision RSD	Inter-day precision RSD	Accuracy Recovery range (n = 3)
Chlorpheniramine maleate (CPM)	50	2	0.9	0.9	99.1-100.6
	100	4	0.4	0.5	
	150	6	0.3	0.4	
Dextromethorphan HBr (DXM)	50	15	0.6	0.5	98.8-100.8
	100	30	0.4	0.4	
	150	45	0.2	1.5	

results remained unaffected by small variations of these parameters.

Assay of tablet dosage form: Assay of tablets data is shown in Table-4.

TABLE-4
ASSAY OF CHLORPHENIRAMINE MALEATE AND DEXTROMETHORPHAN HBr

Drug	Conc. (µg/mL)	Conc. found	% Assay (n = 3)
Chlorpheniramine maleate	4.0000	3.9757	99.7
Dextromethorphan HBr	30.0000	29.2281	98.9

Conclusion

A stability-indicating RP-UPLC method was developed and validated for the determination of dextromethorphan HBr and chlorpheniramine maleate is simple and rapid. Data obtained from precision shows result in term of RSD less than 2 and accuracy for DXM 98.8-100.8% and CPM 99.1-100.6% which conclude that the develop method is reproducible, precise and accurate. Specificity shows no interference of diluent and placebo with main peak. Deliberate changes in chromatographic condition gives RSD value less than 2 which indicate method is robust. Stability study performed in acidic, alkali, oxidative, thermal and photolytic condition. Dextromethorphan hydrobromide and chlorpheniramine maleate are labile in photolytic and oxidative conditions, respectively. Dextromethorphan·HBr and chlorpheniramine maleate are stable in a acidic and photolytic condition, respectively. In all the degradation condition peak purity test pass and degradent peak was well separated from main peak. As there is no interference of excipients at working wavelength; it is fast, with reproducibility and good response. So the method is reliable for the analysis of dextromethorphan·HBr and chlorpheniramine maleate in tablet dosage form with degradants.

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REFERENCES

1. A. Siu and R. Drachtman, Dextromethorphan: A Review of N-methyl-D-aspartate Receptor Antagonist in the Management of Pain, *CNS Drug Rev.*, **13**, 96 (2013); https://doi.org/10.1111/j.1527-3458.2007.00006.x
2. F.E. Simons, Advances in H1-Antihistamines, *N. Engl. J. Med.*, **351**, 2203 (2004); https://doi.org/10.1056/NEJMra033121
3. I.M. Paul, J. Beiler, A. McMonagle, M.L. Shaffer, L. Duda and C.M. Berlin Jr., Effect of Honey, Dextromethorphan, and No Treatment on Nocturnal Cough and Sleep Quality for Coughing Children and Their Parents, *Arch. Pediatr. Adolesc. Med.*, **161**, 1140 (2007); https://doi.org/10.1001/archpedi.161.12.1140
4. P.W. Carr, Xiaoli Wang and D.R. Stoll, The Effect of Pressure, Particle Size and Time on Optimizing Performance in LC, *Anal. Chem.*, 81, 5342 (2009); https://doi.org/10.1021/ac9001244
5. Y. Wu, J.R. Engen and W.B. Hobbins, Ultra Performance Liquid Chromatography (UPLC) Further Improves Hydrogen/Deuterium Exchange Mass Spectrometry, *J. Am. Soc. Mass Spectrom.*, **17**, 163 (2006); https://doi.org/10.1016/j.jasms.2005.10.009
6. G. Chawla and C. Ranjan, Principle, Instrumentation and Applications of UPLC: A Novel Technique of Liquid Chromatography, *Open Chem. J.*, **3**, 1 (2016); https://doi.org/10.2174/1874842201603010001
7. M. Bakshi and S. Singh, Development of Validated Stability-Indicating Assay Methods-Critical Review, *J. Pharm. Biomed. Anal.*, **28**, 1011 (2002); https://doi.org/10.1016/S0731-7085(02)00047-X
8. M. Blessy, R.D. Patel, P.N. Prajapati and Y.K. Agrawal, Development of Forced Degradation and Stability Indicating Studies of Drugs-A Review, *J. Pharm. Anal.*, **4**, 159 (2014); https://doi.org/10.1016/j.jpha.2013.09.003
9. B. Sirigiri, P. Chengalva, S.A. Parameswari and G. Aruna, A Novel HPLC Method for the Simultaneous Determination of Chlorpheniramine

Maleate and Dextromethorphan in Bulk and Pharmaceutical Formulation, *Int. J. Pharm. Sci. Res*., 1447 (2018); https://doi.org/10.13040/IJPSR.0975-8232.9(3).1147-51

10. A.U. Din, H. Shafi, M. Imran, M. Sarwar, M.A. Tahir and S. Khursheed, Validated Reversed-Phase Liquid Chromatographic Method for Simultaneous Determination of Dextromethorphan and Chlorpheniramine in Non-Biological and Biological Matrices Using PDA Detector, *Pharm. Anal. Acta*, **9**, 1 (2018); https://doi.org/10.4172/2153-2435.1000590
11. N. Al-Shaalan, Simultaneous Determination of Chlorphineramine Maleate, Dextromethorphan HBr and Phenylephrin HCl in Codilar Syrup using High Performance Liquid Chromatography, *J. Pharm. Sci. Innov.*, **1**, 29 (2012).
12. S.A. Kumar, M. Debnath and D. Vimala, Simultaneous Estimation of Phenylephrine HCl, Chlorpheniramine Maleate and Dextromethorphan HBr in a Pharmaceutical (Syrup) Formulations by RP-HPLC using PDA detector, *Pak. J. Pharm. Res.*, **2**, 89 (2016); https://doi.org/10.22200/pjpr.2016289-97
13. V. Jain and M.C. Sharma, Validated RP-HPLC Method for Determining the Levels of Bromhexine HCl, Chlorpheniramine Maleate, Dextromethorphan HBr and Guaiphenesin in their Pharmaceutical Dosage Forms, *J. Talibah Univ. Sci.*, **10**, 38 (2016); https://doi.org/10.1016/j.jtusci.2015.02.019)
14. F. Al-Rimawi, Normal-phase LC Method for Simultaneous Analysis of Pseudophedrine Hydrochloride, Dextromethorphan Hydrobromide, Chlorpheniramine Maleate and Paracetamol in Tablet Formulations, *Saudi Pharm. J.*, **18**, 103 (2010); https://doi.org/10.1016/j.jsps.2010.02.006
15. R.S. Latti, N. Jivani, D. Kumar, B. Patel and D. Sheth, Simultaneous Estimation of Paracetamol, Cetrizine Hydrochloride, Chlorpheniramine Maleate and Dextromethorphan Hydrobromide in Solid Dosage form by High Performance Liquid Chromatography. *Inventi Rapid- Pharm Anal. Qual.*. 1024/13 (2013).
16. R. Heydari, A New HPLC Method for the Simultaneous Determination of Acetaminophen, Phenylephrine, Dextromethorphan and Chlorpheniramine in Pharmaceutical Formulations, *Anal. Lett.*, **41**, 965 (2008); https://doi.org/10.1080/00032710801978137
17. T. Goyal, A Novel Stability Indicating HPLC Method for Simultaneous Estimation of Guaiphenesin, Chlorpheneramine Maleate & Dextromethorphan HBr, *Int. J. Pharm. Sci. Res.*, **4**, 4435 (2013); https://doi.org/10.13040/IJPSR.0975-8232.4(11).4435-41
18. K.D. Khalode, S.B. Waikar and S.P. Padmane, A Validated UV Spectrophotometric Method for the Simultaneous Estimation of Dextromethorphan Hydrobromide and Chlorpheniramine Maleate in Syrup Formulation, *Int. J. Pharm. Technol.*, **4**, 4690 (2012).
19. E. Mario and L.G. Meehan, Simultaneous Determination of Nonderivatized Phenylpropanolamine, Glyceryl Guaiacolate, Chlorpheniramine and Dextromethorphan by Gas Chromatography, *J. Pharm. Sci.*, **59**, 538 (1969); https://doi.org/10.1002/jps.2600590421
20. H.M. Heneedaka, S. Ismail, S. Mostafab, E.K. Ehab and M.E. Sadekd, Development and Validation of a Sensitive UHPLC-MS/MS Method for the Simultaneous Analysis of Tramadol, Dextromethorphan Chlorpheniramine and their Major Metabolites in Human Plasma in Forensic Context: Application to Pharmacokinetics, *Biomed. Chromatogr.*, **29**, 998 (2014); https://doi.org/10.1002/bmc.3384
21. Y. Ding, K. Huang, L. Chen, J. Yang, W.-Y. Xu, X.-J. Xu, R. Duan, J. Zhang and Q. He, Simultaneous Quantitative Analysis of Dextromethorphan, Dextrorphan and Chlorphenamine in Human Plasma by Liquid Chromatography-Electrospray Tandem Mass Spectrometry, *Biomed. Chromatogr.*, **28**, 446 (2014); https://doi.org/10.1002/bmc.3054
22. ICH-Harmonized Tripartite Guideline, Stability Testing of New drug Substance and Products Q1A (R2), International Conference on Harmonization, IFPMA, Geneva, Swizerland (2003).
23. ICH-Harmonized Tripartite Guideline, Validation of Analytical Procedures: text and methodology Q2 (R1), International Conference on Harmonization, IFPMA, Geneva,Switzerland (2005).