ORIGINAL ARTICLE

A C G publications *J. Chem. Metrol*. 16:2 (2022) 102-110

journal of chemical metrology

Simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol by HPLC-PDA in pharmaceutical dosage forms[§]

Aykut Kul *

Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, 34452 Istanbul, Türkiye

(Received October 11, 2022; Revised December 03, 2022; Accepted December 08, 2022)

Abstract: A simple and sensitive method has been developed for the simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol by HPLC-PDA in pharmaceutical dosage forms. The separation of the analytes was achieved on an ACE 5 C8, 250 × 4.6 mm, 5 µm column using isocratic elution with a mobile phase containing methanol and 0.1 M phosphoric acid aqueous solution (15:85, v:v) at a flow rate of 1 mL/min. The total run time is 21 min. Chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol were detected at wavelengths (retention times) of 264 nm (9.96 min), 205 nm (6.22 min), 239 nm (19.04 min) and 244 nm (5.22 min), respectively. The injection volume was 10 µL. The assay was in for chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate and paracetamol the concentration ranges 32–48 µg/mL, 9.6–14.4 µg/mL, 32–48 µg/mL, 104–156 µg/mL, respectively. LOQs (µg/mL) and LODs (µg/mL) 1.38 and 0.46 for chlorpheniramine maleate, 0.05 and 0.02 for pseudoephedrine hydrochloride, 0.76 and 0.25 for oxolamine citrate, 0.21 and 0.07 for paracetamol, respectively. Recoveries of the analytes were between 98% and 102% with intra-and inter-day precisions (as relative standard deviation) of ≤2%. In addition, expanded uncertainty values were less than 2 for all analytes. Method validation was carried out according to ICH guideline Q2 (R1). The analytical method validated was successfully applied to pharmaceutical dosage forms.

Keywords: Chlorpheniramine maleate; pseudoephedrine hydrochloride; oxolamine citrate; paracetamol; pharmaceutical dosage forms; HPLC-PDA.

1. Introduction

Chlorpheniramine maleate is an antihistamine used to treat symptoms of allergic conditions [1]. Pseudoephedrine hydrochloride relieves nasal congestion associated with colds or allergies [2]. Oxolamine Citrate is used as a cough suppressant in the treatment of pharyngitis, bronchitis, and whooping cough [3]. Paracetamol is a drug active ingredient with analgesic and antipyretic effects [4].

Medicines containing chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol (Figure 1) are frequently used in clinical procedures as antipyretic and analgesic agents to treat pain and fever associated with cold and flu [5-7].

* Corresponding author E-Mail: aykut.kul@istanbul.edu.tr

[§]This study has also been presented as an oral presentation at 10. National Analytical Chemistry Congress at Muğla Sıtkı Koçman University, Muğla, Türkiye on September 08 – 11, 2022

The article was published by ACG Publications
http://www.acgpubs.org/journal/journal-of-chemical-metrology July-December 2022 EISSN:1307-6183
DOI:http://doi.org/10.25135/jcm.752210.2596
Available online: December 15, 2022

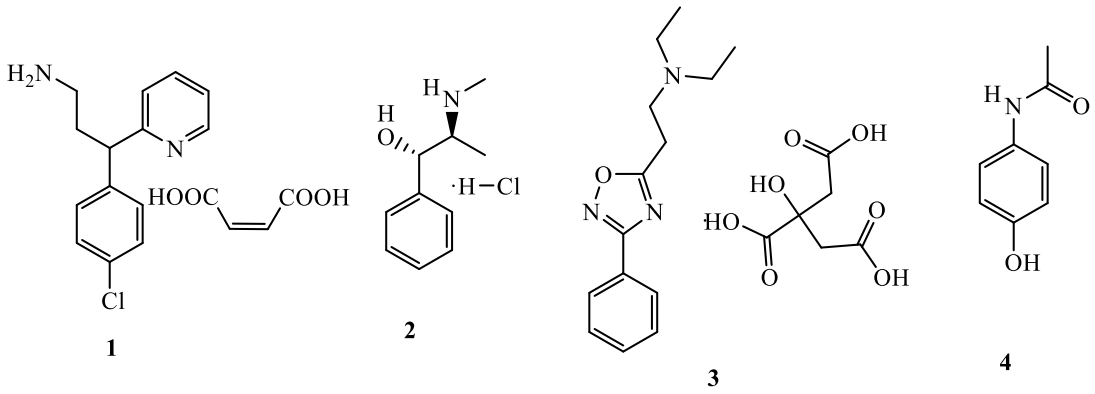

Figure 1. Chemical structures of chlorpheniramine maleate (**1**), pseudoephedrine hydrochloride (**2**), oxolamine citrate (**3**), and paracetamol (**4**)

In the literature, it has been revealed that chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol have been determined in a single form in combination with other drugs [8-15]. However, as far as I know, no study has been found for the simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol. Thus, this study aimed to develop a simple and selective HPLC-PDA method for the simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol in pharmaceutical dosage forms.

2. Experimental

2.1. Materials

The pharmaceutical preparation of chlorpheniramine maleate (0717115, 99.5%w/w), pseudoephedrine hydrochloride (209417, 99.5%w/w), oxolamine citrate (0180717M, 99.7%w/w), and paracetamol (E32005038, 99.5%w/w), reference standards and tablet (Katarin Forte® tablet containing 4 mg chlorpheniramine maleate, 60 mg pseudoephedrine hydrochloride, 200 mg oxolamine citrate, and 650 mg paracetamol) was provided from Biofarma (Türkiye). Other chemicals of analytical quality were supplied by Merck (Germany). An Elga Milli-Q water (Bedford, MA, UK) was used for purification to produce HPLC-grade water.

2.2. Standard Solutions

Standard solution mixtures were prepared at 7 different concentrations (80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120%). This mixture contained chlorpheniramine maleate (32, 36, 38, 40, 42, 44, and 48 µg/mL), pseudoephedrine hydrochloride (9.6, 10.8, 11.4, 12, 12.6, 13.2, and 14.4 µg/mL), oxolamine citrate (32, 36, 38, 40, 42, 44, and 48 µg/mL) and paracetamol (104, 117, 123.5, 130, 136.5, 143, and 156 µg/mL)

2.3. HPLC-PDA Conditions

The chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol were analyzed by using a Shimadzu LC 20A liquid chromatographic system (Shimadzu, Kyoto, Japan) with an SPD-M20A photodiode array detector. The chromatographic separation was achieved on an ACE 5 C8, 250 × 4.6 mm, 5 µm column with a mobile phase containing methanol (mobile phase A) and 0.1 M phosphoric acid aqueous solution (mobile phase B) (15:85, v:v) at a flow rate of 1 mL/min. The run time was 21.0 minutes. Chlorpheniramine maleate, pseudoephedrine hydrochloride,

oxolamine citrate, and paracetamol were detected at wavelengths of 264 nm, 205 nm, 239 nm, and 244 nm, respectively.

2.4. Method Validation

This method developed for the simultaneous analysis of the chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol has been validated according to the ICH guidelines [16]. System suitability, specificity, accuracy, precision sensitivity, linearity, stability, and robustness parameters were evaluated throughout the validation study.

2.4.1. System Suitability

For system suitability testing, a sample containing chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol was prepared and analyzed in six replicates. The theoretical plate value, resolution, and tailing factor assess system suitability.

2.4.2. Specificity

The specificity is the ability to assess unequivocally the analyte in the presence of components. The specificity of the developed HPLC method was determined by injecting a blank (solvent), placebo, and standard mixture solution.

2.4.3. Accuracy

The accuracy is the closeness of agreement between a measured quantity value and a true quantity value of a measurand. The accuracy was evaluated with three different levels (32, 40, and 48 µg/mL for chlorpheniramine maleate, 9.6, 12, and 14.4 µg/mL for pseudoephedrine hydrochloride, 32, 40, and 48 µg/mL for oxolamine citrate and 104, 130, and 156 µg/mL for paracetamol) of replicate samples (in placebo) on three different days. The recovery was calculated using the formula below. Acceptance limits for recovery are in the range of 98-102%.

%Recovery = Recovered concentration /Injected concentration x 100

2.4.4. Precision

Three different concentrations (32, 40, and 48 µg/mL for chlorpheniramine maleate, 9.6, 12, and 14.4 µg/mL for pseudoephedrine hydrochloride, 32, 40, and 48 µg/mL for oxolamine citrate and 104, 130, and 156 µg/mL for paracetamol) were used in intra-day and inter-day precision studies. For the precision study, all concentration levels were analyzed 6 times on 3 different days. The relative standard deviation (RSD) was calculated using the formula below. Acceptance limits for % RSD should be less than 2%.

%Relative standard deviation = Standard deviation/Mean x 100

2.4.5. Detection and Quantitation Limits

The limit of quantification (LOQ) of the method was determined when the signal-to-noise (S/N) ratio was 10, and the limit of detection (LOD) of the method was determined when the S/N ratio was 3. The repeatability of the LOD and LOQ values was evaluated by 6 injections and calculating the RSD values.

2.4.6. Linearity

The linearity of the method is evaluated by the addition of 7 different concentrations in the range of 32–48 µg/mL for chlorpheniramine maleate, 9.6–14.4 µg/mL for pseudoephedrine hydrochloride, 32–

48 µg/mL for oxolamine citrate, and 104–156 µg/mL for paracetamol. Three calibration curves were performed for each analyte.

2.4.7. Stability

To determine the stability of the method, the freshly prepared standard sample and the sample prepared from the tablet were kept for 24 hours and injected once.

2.4.8. Robustness

The analysis method's robustness refers to detecting the target without being affected when the test parameters are intentionally slightly changed. The changes in mobile phase flow rate and mobile phase composition were examined to investigate the influence of a slight change in method parameters on the detection.

2.5. Sample Preparation of Tablets

Twenty tablets containing chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol were individually weighed into a 100 mL flask and dissolved in ~90 mL mobile phases. The contents of the flask were sonicated for 30 minutes and a mobile phase was added to bring the volume to 100 mL. The sample was then mixed and filtered. The filtrate was transferred to a vial for chlorpheniramine maleate analysis. Then, the filtrate was diluted 50 times and given to a vial for the determination of pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol. The prepared samples were analyzed in the HPLC system.

2.6. Uncertainty Assessment

The main uncertainty contributions of the validated method were calculated according to the EURACHEM guide. The uncertainty from the purity of the compound ($u_{standard}$), weighing ($u_{weighing}$), calibration curve parameters ($u_{calibration}$), recovery ($u_{recovery}$), and repeatability ($u_{repeatability}$) were evaluated for the main uncertainty contributions. The combined uncertainty ($U_{Combined}$) was calculated using the formula below

$$u = \sqrt{(u_{standard})^2+(u_{weighing})^2\ (u_{calibration})^2+(u_{recovery})^2+(u_{repeatability})^2}$$

The expanded uncertainty ($U_{Expanded}$) was calculated at a 95% confidence level by multiplying the combined uncertainty with the coverage factor (k=2). The methodology applied for the estimation of the uncertainty budget is given in the literature, especially in the EURACHEM Guide [16-22]. More details are not written here to avoid repetition.

3. Results and discussion

3.1. Chromatographic Separation

In this study, different types of analytical columns were tested, including C8, CN, C18, PFPP, biphenyl, and phenyl, with different mobile phases such as the mixtures of methanol or acetonitrile with ortho-phosphoric acid, acetic acid, and formic acid solutions. A new method was successfully developed for the simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol by HPLC-PDA in pharmaceutical dosage forms with a superior resolution for the isolated peaks.

3.2. Validation of the Method

3.2.1. System suitability

The chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol eluted at 9.96, 6.22, 19.04, and 5.22 minutes, respectively, tailing factor for peak less than 2 indicates good peak symmetry, and theoretical plates greater than 2000 ensure column efficacy. Results are represented in Table 1.

Table 1. System suitability results

Parameter	Criteria	Chlorpheniramine Maleate	Pseudoephedrine Hydrochloride	Oxolamine Citrate	Paracetamol
Tailing factor (T)	T < 2	0.975	1.03	0.88	1.11
Theoretical plates (N)	N > 2000	9481	10779	11310	11805
Resolution factor (Rs)	Rs > 2	12.03	4.59	16.39	9.39

3.2.2. Specificity

The method exhibited the absence of endogenous interferences at the retention time of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol (Figure 2), and all analyte peaks were pure, with a mean peak purity index value of 0.99, obtained from the photodiode array detector.

3.2.3. Accuracy

The accuracy of the method was determined for all analytes at 3 different concentrations. The results are given as a percentage of the difference between added and measured concentrations (Table 2).

Table 2. The data of the developed method

Parameter	Chlorpheniramine Maleate	Pseudoephedrine Hydrochloride	Oxolamine Citrate	Paracetamol
Calibration Equation*	y = 14541x - 12926	y = 85336x - 14986	y = 18602x - 58729	y = 15406x - 44506
The Determination	0.991	0.998	0.999	0.992
Linearity Range	32-48	9.6-14.4	32-48	104-156
Number of Points	7	7	7	7
LOQ (µg/mL)	1.38	0.05	0.76	0.21
LOD (µg/mL)	0.46	0.02	0.25	0.07
Accuracy (%)	98.14-101.56	98.35-101.34	98.65-101.21	99.33-101.55
Repeatability (%RSD)	0.55-0.80	0.49-1.33	0.57-1.60	0.31-0.74
Intraday Precision	0.52-1.01	0.50-0.84	0.23-0.98	0.39-0.58
Interday Precision	1.12-1.67	0.51-0.78	0.87-1.08	0.66-0.82

* Linear regression analysis with a calibration equation of y = ax + b in which x is the concentration in µg/mL the compound and y is the peak area.

3.2.4. Precision

Relative standard deviation (RSD) values calculated for repeatability and reproducibility studies are given in Table 2. These results show that the method has the required sensitivity.

3.2.5. LOQ, LOD, and Linearity

LOQ, LOD, linearity of the method, calibration equation, and correlation coefficient of determination are shown in Table 2.

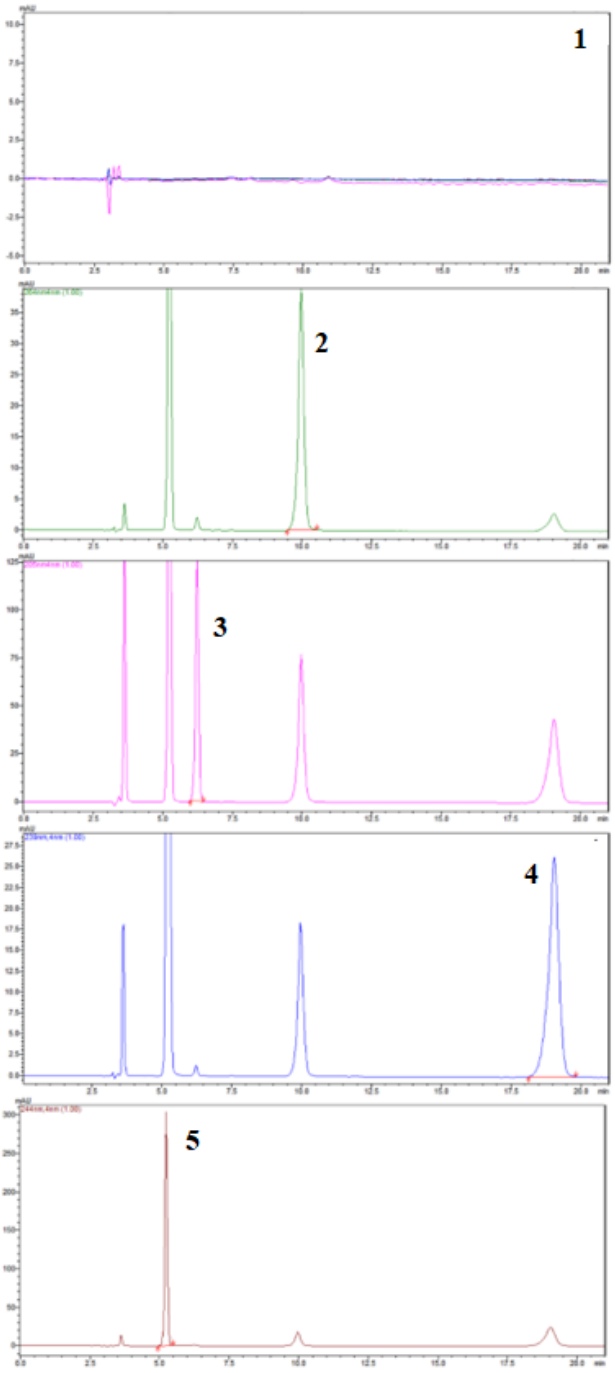

Figure 2. Chromatograms of placebo (**1**), chlorpheniramine maleate (**2**), pseudoephedrine hydrochloride (**3**), oxolamine citrate (**4**), and paracetamol (**5**)

3.2.6. Stability

According to the results obtained, an acceptable change was not observed in the applied method. Therefore, stability was not considered a critical parameter of the method (Table 3).

Table 3. Result of stability study

Parameter	Chlorpheniramine Maleate	Pseudoephedrine Hydrochloride	Oxolamine Citrate	Paracetamol
Time (h)	Area	Area	Area	Area
0	554127	997063	679912	1896378
24	550102	979798	662586	1875682
%RSD	0.52	1.24	1.83	0.78

3.2.7. Robustness

Significant changes are not observed in assay results upon small variations in the chromatographic conditions, flow rate, or mobile phase composition (Table 4).

Table 4. Result of the robustness study

Parameter	Chlorpheniramine Maleate	Pseudoephedrine Hydrochloride	Oxolamine Citrate	Paracetamol
Flow rate mL/min	**Recovery (%)**	**Recovery (%)**	**Recovery (%)**	**Recovery (%)**
0.9	98.25	99.42	98.76	99.53
1.0	99.36	99.81	98.21	100.92
1.1	99.16	100.58	101.1	100.63
%RSD	0.60	0.59	1.54	0.73
Mobile phase (A:B)	**Recovery (%)**	**Recovery (%)**	**Recovery (%)**	**Recovery (%)**
14:86	98.39	100.12	101.2	99.53
15:85	99.25	100.96	99.41	99.26
16:84	98.96	99.92	98.93	98.87
%RSD	0.44	0.55	1.20	0.33

3.3. Application to the Analysis of Pharmaceutical Dosage Forms

The proposed HPLC method was applied to the simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol in Katarin Forte®. The quantitative results of these assays are summarized in Table 5.

Table 5. Result of pharmaceutical dosage forms

Analytes	Labeled amount (mg)	Found amount (mg)	Found amount (%)
Chlorpheniramine Maleate	4.00	4.02	100.50
Pseudoephedrine Hydrochloride	60.00	60.06	100.10
Oxolamine Citrate	200.00	200.03	100.02
Paracetamol	650.00	650.10	100.02

3.4. Uncertainty Assessment

The combined and expanded uncertainty value is given the Table 6. In addition, detailed calculations are given in the supporting information file. Since the uncertainty value from the $u_{weighing}$ parameter is very small, it is not given in the table and has been neglected in the calculation.

Table 6. Data of combined and expanded uncertainty

Analytes	$u_{standard}$	$u_{calibration}$	$u_{wecovery}$	$u_{repeatability}$	$u_{combined}$	$U_{expanded}$ (k=1.96)
Chlorpheniramine Maleate[a]	0.29	0.62	0.21	0.22	0.75	1.47
Pseudoephedrine Hydrochloride[b]	0.29	0.38	0.30	0.44	0.71	1.39
Oxolamine Citrate[c]	0.17	0.48	0.09	0.65	0.84	1.64
Paracetamol[d]	0.29	0.35	0.15	0.25	0.54	1.06

[a]Uncertainty for 39.94 µg/mL; 95 % confidence level; U % values reported.
[b]Uncertainty for 11.97 µg/mL; 95 % confidence level; U % values reported.
[c]Uncertainty for 39.99 µg/mL; 95 % confidence level; U % values reported.
[d]Uncertainty for 130.03 µg/mL; 95 % confidence level; U % values reported.

4. Conclusions

A new HPLC-PDA method has been developed for the simultaneous determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol. The low % RSD value of precision, robustness, and other validation parameter was found which indicates the suitability of this method for routine quantitative and qualitative determination of chlorpheniramine maleate, pseudoephedrine hydrochloride, oxolamine citrate, and paracetamol pharmaceutical dosage forms. In addition, expanded uncertainty values were less than 2 for all analytes. The statistical analysis of data found proves that the proposed RP-HPLC method is specific, sensitive, accurate, robust, reproducible, and precise. The developed and validated method is useful for the routine determination the those measurands and their stability tests of in the combined formulation of the drugs by using same solvent system and setup.

ORCID
Aykut Kul: 0000-0002-1195-0939

References

[1] M. Maithani, R. Raturi, V. Gautam, D. Kumar, A.K. Chaudhary, A. Gaurav and R. Singh (2010). Development and validation of an RP-HPLC method for the determination of chlorpheniramine maleate and phenylephrine in pharmaceutical dosage form, *J. Compr. Pharm.* **1**, 1-4.

[2] D. De Orsi, L. Gagliardi, A. Bolasco and D. Tonelli (1996). Simultaneous determination of triprolidine, pseudoephedrine, paracetamol,l and dextromethorphan by HPLC, *Chromatographia* **43**, 496-500.

[3] L. Kirilmaz, A Kendirci and T. Güneri (1991). Sustained-release dosage form of oxolamine citrate: preparation and release kinetics, *J. Microencapsul.* **9**, 167-172.

[4] W.F. Bynum (2008). History of medicine: a very short introduction. Oxford: Oxford University Press. ISBN 978-0199215430.

[5] G.G.G. De Oliveira, A. Feitosa, K. Loureiro, A.R. Fernandes, E.B. Souto and P. Severino (2017). Compatibility study of paracetamol, chlorpheniramine maleat,e and phenylephrine hydrochloride in physical mixtures, *Saudi Pharm. J.* **25**, 99-103.

[6] O. Pirol, M. Sukuroglu and T. Ozden (2011). Simultaneous determination of paracetamol, phenylephrine hydrochloride, oxolamine citrate, and chlorpheniramine maleate by HPLC in pharmaceutical dosage forms, *Electron. J. Chem.* **8**, 1275-1279.

[7] M.J. Akhtar, S. Khan and M. Hafiz, (1994). High-performance liquid chromatographic assay for the determination of paracetamol, pseudoephedrine hydrochloride, and triprolidine hydrochloride, *J. Pharm. Biomed. Anal.***12**, 379-382.

[8] A. Acheampong, W.O. Gyasi, G. Darko, J. Apau and S. Addai-Arhin, (2016). Validated RP-HPLC method for simultaneous determination and quantification of chlorpheniramine maleate, paracetamol and caffeine in tablet formulation, *SpringerPlus* **5**, 1-8.

[9] M.L. Qi, P. Wang, Y.X. Leng, J.L. Gu and R.N. Fu (2002). Simple HPLC method for simultaneous determination of acetaminophen, caffeine and chlorpheniramine maleate in tablet formulations, *Chromatographia* **56**, 295-298.

[10] S. Karakuş, İ. Küçükgüzel and Ş.G. Küçükgüzel (2008). Development and validation of a rapid RP-HPLC method for the determination of cetirizine or fexofenadine with pseudoephedrine in binary pharmaceutical dosage forms, *J. Pharm. Biomed. Anal.* **46**, 295-302.

[11] F. Ibrahim, N. El-Enany, R.N. El-Shaheny and I.E. Mikhail, (2015). Development and validation of a new HPLC method for the simultaneous determination of paracetamol, ascorbic acid, and pseudoephedrine HCl in their co-formulated tablets. Application to in vitro dissolution testing, *Anal. Sci.* **31**, 943-947.

[12] R.V. Rele and S.P. Patil, (2011). Reversed phase high pressure liquid chromatography technique for determination of oxolamine citrate from pharmaceutical formulation, *Res. J. Pharm. Technol.* **4**, 787-789.

[13] P. R. Battu and M.S. Reddy (2009). RP-HPLC method for simultaneous estimation of paracetamol and ibuprofen in tablets, *Asian J. Research. Chem.* **2**, 70-72.

[14] M. L. Altun (2002). HPLC method for the analysis of paracetamol, caffeine and dipyrone, *Turk. J. Chem.* **26**, 521-528.

[15] T. Belal, T. Awad and R. Clark (2009). Determination of paracetamol and tramadol hydrochloride in pharmaceutical mixture using HPLC and GC-MS. *J. Chromatogr. Sci.* **47**, 849-854.

[16] H. Kiziltas, A.C. Goren, Z. Bingöl, S.H. Alwasel and I. Gulcin (2021). Anticholinergic, antidiabetic and antioxidant activities of *Ferula orientalis* L. determination of its polyphenol contents by LC-HRMS, *Rec. Nat. Prod.* **15**, 513-528.

[17] M. Topal (2020). Secondary metabolites of ethanol extracts of *Pinus sylvestris* cones from eastern Anatolia and their antioxidant, cholinesterase and alpha-glucosidase activities, *Rec. Nat. Prod.* **14**, 129-138

[18] A.C. Gören, G. Bilsel and M. Bilsel. (2007). Rapid and simultaneous determination of 25-OH-vitamin D 2 and D 3 in human serum by LC/MS/MS: Validation and uncertainty assessment, *J. Chem. Metrol*. **1**, 1-9

[19] H. Kızıltaş, Z. Bingöl, A.C. Gören, S.M. Pinar, S.H. Alwasel and İ. Gülçin, (2021). LC-HRMS profiling of phytochemicals, antidiabetic, anticholinergic and antioxidant activities of evaporated ethanol extract of *Astragalus brachycalyx* Fischer, *J. Chem. Metrol*. **15**, 135-151.

[20] Z. Ozer, S. Carikçi, H. yılmaz, T. Kılıç, T. Dirmenci and A.C. Goren (2020). Determination of secondary metabolites of *Origanum vulgare* subsp. *hirtum* and *O. vulgare* subsp. *vulgare* by LC-MS/MS, *J. Chem. Metrol*. **14**, 35-34.

[21] B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The fitness for purpose of analytical methods – a laboratory guide to method validation and related topics, (2nd ed. 2014). ISBN 978-91-87461-59-0

[22] International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use ICH harmonized tripartite guideline validation of analytical procedures: text and methodology Q2(R1) Validation. https://www.ich.org/page/quality-guidelines\