**Validated RP-HPLC and TLC-densitometric Methods for Analysis of Ternary Mixture of Cetylpyridinium Chloride, Chlorocresol, and Lidocaine in Oral Antiseptic Formulation**

**Nada S. Abdelwahab(a), Nouruddin W. Ali(a), M. Abdelkawy(b) and Aml A. Emam(a)**\*

**a) Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Al shaheed Shehata Ahmed Hegazy st., 62514.Beni-Suef, Egypt.**

**b) Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini st.,11562, Cairo, Egypt.**

\***Corresponding author**

* e-mail address: aml17484@gmail.com
* Mobile : +2 01092109219
* Fax : +2 082/2344535

**Abstract**

This work was concerned with development, optimization, application, and validation of RP-HPLC and TLC-densitometric chromatographic methods for analysis of cetylpyridinium chloride, chlorocresol, and lidocaine in Canyon® gel. The firstly developed RP-HPLC method depended on chromatographic separation on a ZORBAX Eclipse Plus, C8 column, elution with a mobile phase consisted of 0.05% phosphoric acid solution: acetonitrile: methanol (15: 24: 61, by volume), pumping the mobile phase at a flow rate of 1.00 ml min-1 with UV detection at 220 nm. While in the secondly developed chromatographic method, TLC-densitometric method, complete separation of the studied mixture was achieved using methanol: acetone: acetic acid (7: 3: 0.2, by volume) as a mobile phase, aluminum plates precoated with silica gel 60 F254 as a stationary phase, and 215 nm as a scanning wavelength. Factors affecting the developed methods were studied and optimized, moreover, methods had been validated as per ICH guideline and the results indicated that the suggested methods were reproducible, reliable, and applicable for rapid routine analysis. Statistical comparison of the two developed methods with the reported HPLC ones using F and student's-t tests showed no significant difference.

**Keywords:**

Cetylpyridinium chloride; Chlorocresol; Lidocaine; RP-HPLC; TLC-densitometry.

**Introduction**

Cetylpyridinium chloride (CE) is chemically designated as 1-hexadecylpyridinium chloride, (**1, 2)**, (Figure 1a). It is an antiseptic detergent (**1)**; a cationic quaternary ammonium compound used in some types of mouthwashes, toothpastes, lozenges, throat, breath and nasal sprays. It is effective in preventing dental plaque and reducing gingivitis **(3, 4)**. Chlorocresol (CH) is chemically designated as 4-chloro-3-methylphenol, **(1, 2)**, (Figure 1b). It is an antimicrobial preservative. Lidocaine (LI), is chemically designated as 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide, **(1, 2),** (Figure 1c). LI is a common local anesthetic and anti-arrhythmic drug **(5)**. It is used topically to relieve itching, burning, and pain from skin inflammations **(6)** and as a local anesthetic for minor surgery **(5-8)**. It is characterized by rapid onset of action and intermediate duration of efficacy **(9-11)**. A combination of CE, CH, and LI works to relieve pain caused by teething, mouth ulcers, or denture irritation. Antiseptics help to prevent the irritated areas from getting infected leading to fast healing.

CE, CH, and LI were analyzed separately by titrimetric methods **(1)**, while only LI was determined in USP **(2)** by HPLC method.The literature survey revealed several analytical methods for determination of CE either alone or in combination with other drugs such as spectroscopic, **(12-15)**,chromatographic, **(16-22)**, and electrochemical methods, **(23-25)**. Different methods were reported for determination of CH such as spectroscopic, **(26)**,and chromatographic, **(27-34)**,methods. Also LI was analyzed by different spectroscopic, **(35-37)**, chromatographic, **(6, 35, 38-51)**, and electrochemical methods, **(8, 10)**. Only one HPLC-DAD method was reported for determination of CE along with LI in oral gel dosage forms **(51)** but no methods were reported for determination of CE, CH, and LI in their ternary mixture and in combined dosage forms.

In the reported HPLC-DAD method **(51)**, in spite of its stability indicating properties, it separated the two drugs, CE and LI, by gradient elution after 8 minutes and detected them at two different wavelengths. So it was important to validate simple analytical methods with short analysis time for simultaneous determination of CE, CH, and LI in their ternary mixture and combined dosage forms. Hence the work in this manuscript aimed to introduce two selective, accurate and precise chromatographic methods for the first time that can be applied for simultaneous determination of CE, CH, and LI ternary mixture and then validate the introduced methods according to ICH guidelines **(52)**.

**Instruments**

***RP-HPLC method***

HPLC instrument (Agilent Technologies, Waldbronn, Germany) equipped with a preparative pump G 1361A (Agilent Technologies, Waldbronn, Germany), a diode array detector VL, G 1315D (Agilent Technologies, Waldbronn, Germany), a thermostated column compartment G 1316A (Agilent Technologies, Waldbronn, Germany), and a preparative Auto sampler G 2260A (Agilent Technologies, Waldbronn, Germany). Separation and quantification were carried out on C8 column (Agilent Technologies, ZORBAX Eclipse Plus, New York, USA) (25cm × 4.6 mm i. d, 5 µm particle size).

***HPTLC-densitometric method***

The instruments used in this study included a TLC Scanner 3 densitometer (Camag, Muttenz, Switzerland) controlled by WINCATS software (version 3.15) (Camag, Muttenz, Switzerland); a sample applicator for TLC Linomat V equipped with a 100 mL syringe (Camag, Muttenz, Switzerland); an ultraviolet (UV) lamp with short wavelength of 254 nm (VL-6.LC; Marne la Vallee, France); TLC plates (20 x 10 cm) coated with 60 F254 silica gel (Merck, Darmstadt, Germany) with 0.2 mm thickness. During TLC scanning, the scanning mode was absorbance, source of radiation was a deuterium lamp, the slit dimensions were adjusted to 6 x 0.45 mm, and the scanning speed was 20 mm.s-1.

**Materials and reagents**

***Pure standards***

Cetylpyridinium chloride (CE), chlorocresol (CH), and lidocaine (LI) pure standards were kindly supplied by Bioregional international group for phoenic for advanced products, 6 EL Mosheer Ahmed Ismail Street. Massken Sheraton, Cairo, with claimed purity of 98.53, 97.94, and 99.96 %, respectively according to the manufacturer certificate.

***Pharmaceutical formulations***

Canyon® Gel: Batch No. 100004 manufactured by Bioregional international group for phoenic for advanced products and labeled to contain 0.2, 1, and 10 mg CE, CH, LI, respectively per gram.

***Chemicals and solvent***

All chemicals used throughout this work were of analytical grade and were used without further purification; deionized water (SEDICO pharmaceutical Co., 6th October City, Cairo, Egypt), methanol, acetone, phosphoric acid (El-Nasr Pharmaceutical Chemicals Co., Abu Zabaal, Cairo, Egypt). Acetic acid, methanol, and acetonitrile HPLC grade (Chromosolve®, Sigma-Aldrich, Chemie GmbH, Germany - supplied by the Egyptian International Center for Import, Cairo, Egypt).

***Solutions***

*Stock standard solutions* of CE, CH, and LI (S = 1 mg mL-1): 100 mg of CE, CH, and LI were accurately and separately weighed into three separate 100-mL volumetric flasks and the volume was then completed to the mark with methanol.

*Working standard solutions* of CE, CH, and LI (W= 0.1 mg mL-1): They were prepared by diluting 10 mL from their respective stock solutions (S = 1 mg mL-1) into three separate 100-mL volumetric flasks and the volume was completed using methanol.

*Pharmaceutical dosage form solution* (D = 0.02, 0.1, and 1 mg mL-1 for CE, CH, and LI, respectively): 10 gm Canyon® gel were emptied and triturated well in a mortar. A sample containing CE, CH, and LI equivalent to 0.2, 1, and 10 mg, respectively was transferred into 10- mL volumetric flask. About 7 mL methanol was added and the flask was sonicated for 15 min. The solution was filtered and the volume was completed to the mark with methanol to obtain stock sample solution (D) containing 0.02, 0.1, and 1 mg mL-1 for CE, CH, and LI, respectively.

**Procedure**

***Chromatographic conditions***

***RP-HPLC method:*** Chromatographic separation was performed on C8 column (Agilent Technologies, ZORBAX Eclipse Plus, New York, USA) (25cm × 4.6 mm i. d, 5 µm particle size) using a mobile phase of 0.05% phosphoric acid solution: acetonitrile: methanol (15: 24: 61, by volume) delivered at a flow rate of 1 mL min-1, samples were injected in volumes of 20 µL at ambient temperature, and monitored at 220 nm using DAD then peak areas were recorded.

***TLC-densitometric method:*** Samples were applied on aluminum plates precoated with silica gel 60 F254 (20 x 10 cm) as bands of 6 mm width with a 100 µL sample syringe using an auto-sampler. The space between bands was 8.9 mm and a constant application rate of 0.1µL/s was used. The scanning speed was 20 mm/s and the slit dimension was 6.0 x 0.3 µm. The mobile phase consisted of methanol: acetone: acetic acid (7: 3: 0.2, by volume). A glass chamber saturated with the mobile phase was used for linear ascending development. Development of the plates was allowed till the mobile phase migrates to a distance of 8 cm. Following the development, the plates were air dried. Densitometric scanning was performed using CAMAG TLC Scanner in the reflectance-absorbance mode at 215 nm and operated by WINCATS software using deuterium lamp as a radiation source then peak areas were recorded.

***Linearity and construction of calibration curves***

External standards were used to construct calibration curves for the two proposed methods because using of an external standard allows analysis of a series of samples using a single calibration curve which is important advantage when we have many samples to be analyzed **(53)**.

***RP-HPLC method:*** Calibration graphs for CE, CH, and LI were constructed by recording and storing the peak area of different concentrations of each in the ranges of 1 – 30 µg mL-1, 0.5 - 30 µg mL-1, and 1 - 50 µg mL-1, respectivelyprepared by suitable dilutions of their respective working standard solutions (W = 0.1 mg mL-1) then a volume of 20 μL of each solution was separately injected in triplicates. The procedure under chromatographic conditions was then followed. Calibration graphs were constructed by plotting peak area ratios (using a concentration of 10 µg mL-1 of each drug as an external standard to its respective calibration curve) versus the corresponding concentration and regression equations were then computed.

***TLC-densitometric method:***  Accurate volumes of 0.1-5, 0.2-3, and 0.4-6 mL were transferred from CE, CH, and LI stock standard solutions (S = 1 mg mL-1), respectively into three separate sets of 10 mL volumetric flasks; the volumes were completed to the mark with methanol. A volume of 10-µL from each flask was spotted in triplicates on TLC plates. The procedure under chromatographic conditions was performed. The peak area ratios (using 1µg band-1each of CE, CH, and LI as external standards) were plotted against their respective concentrations to obtain the calibration graphs.

***Application to pharmaceutical formulations***

***RP-HPLC method:*** Four sample solutions, D1, D2, D3, and D4 were prepared from Canyon® gel stock sample solution (D) by accurately transferring 1 mL, 0.2 mL, 0.02, and 0.5, respectively into four separate 10 mL volumetric flasks and the volume was completed to the mark with methanol. 20 µL of the last prepared solutions were injected in triplicates, for each, following the procedure described for the chromatographic conditions. Concentrations of CE in D1 and D4 sample, CH in D2 and D4 sample, and LI in D3 and D4 sample solutions were then calculated from the respective constructed regression equations. Standard addition technique was carried out to prove the accuracy of the suggested method, and it was performed by spiking the pre-analyzed CE, CH, and LI samples in D1, D2, and D3, respectively (2 µg mL-1 for each) with an extra 50 %, 100 %, 150 % and 200 % of standard CE, CH, and LI.

***TLC-densitometric method:***  A sample of 5 µL of Canyon® gel solution (D) containing 0.02, 0.1, and 1 mg mL-1 of CE, CH, and LI, respectively was spotted on TLC plates in triplicate following the procedure described for the chromatographic conditions to obtain concentrations of (0.1, 0.5, and 5 µg band-1, respectively) and chromatographic method was then continued as before. The peak area ratio of each spot was determined and the concentrations were calculated from the respective previously computed regression equations. Standard addition technique was carried out to ensure accuracy of the suggested method, and it was performed by spiking the pre-analyzed CE, CH, and LI samples (0.1, 0.5, and 5 µg band-1, respectively) with an extra standard CE, CH, and LI, respectively.

**Results**

Although different methods were reported for determining each of CE, CH, and LI either alone or with other drugs, none of these methods determined CE, CH, and LI in their ternary mixture. The main task of this work was to develop sensitive, selective and accurate RP-HPLC and TLC chromatographic methods for determination of CE, CH, and LI in their ternary mixture and in pharmaceutical formulations, with satisfactory precision for good analytical practice (GAP).

The proposed methods were applied for determination of CE, CH, and LI in their pharmaceutical formulation. Firstly, for RP-HPLC method, calibration curves relating the peak area ratios versus the corresponding concentrations in the ranges of 1-30, 0.5-30, and 1-50 µg mL-1 for CE, CH, and LI, respectively (using 10 µg mL-1 of each drug as a respective external standard). The concentrations of CE, CH, and LI were calculated from the corresponding regression equations. Regression equation parameters were given in (Table I). Secondly, for TLC-densitometric method, calibration curves relating the peak area ratios versus the corresponding concentrations in the ranges of 0.1-5, 0.2-3, and 0.4-6 µg band-1 for CE, CH, and LI, respectively (using a concentration of 1 µg band-1 of CE, CH, and LI as an external standard). The concentrations of CE, CH, and LI were calculated from the corresponding regression equations. Regression equation parameters were given in (Table I)

In order to evaluate the applicability of the proposed methods, the methods were applied to Canyon® gel. Three peaks were detected at tR = 3.7 ± 0.2, 2.7 ± 0.1, and 2.1 ± 0.1 min for RP-HPLC method and at Rf = 0.12 ± 0.01, 0.81 ± 0.02, and 0.48 ± 0.01 for TLC-densitometric method for CE, CH, and LI, respectively indicating no interference from the excipients that routinely occur in gel pharmaceutical formulations. The mean % recovery of the drug content was found to be acceptable, (Table II).

The suggested methods were compared favorably with the reported HPLC methods **(51, 29)** as shown from the values of the calculated Student's-t and F-values, confirming that there was no significant difference within a probability of 95% between the proposed methods and the reported ones (Table III).

**Discussion**

**Methods development and optimization**

***Optimization of RP-HPLC method***

*Optimization of mobile phase:* Several developing systems were tried; water: methanol, water: acetonitrile with different ratios at different flow rates. Water was replaced with phosphoric acid solution at different strengths and with different ratios. Finally, a mobile phase of 0.05% phosphoric acid solution: acetonitrile: methanol (15: 24: 61, by volume), a stationary phase of ZORBAX Eclipse Plus C8 column, a flow rate of 1.00 ml min-1, and UV detection at 220 nm resulted in stable baseline, adequate separation and sharp peaks in a suitable analysis time where tR values for CE, CH, and LI were 3.7 ± 0.2, 2.7 ± 0.1, and 2.1 ± 0.1 min, respectively.(Figures 2, 3)**.**

*Selection of stationary phase:* ZORBAX Eclipse plus C18 and C8 columns were tried, whereas C8 column provided higher resolution and more symmetric peaks.

*Selection of scanning wavelength:* Different wavelengths were tried where 220 nm provided the best results with respect to peak sensitivity.

*Optimization of column temperature:* The thermo stated column compartment was adjusted to different temperatures (20, 25 and 30 oC). Note that; the column temperature neither affected the peak sharpness nor the chromatographic separation.

*Optimization of flow rate:* Different flow rates were tried and the best flow rate was 1 mL min-1.

A satisfactory separation with high peaks symmetry were obtained using the above optimum conditions where the tR values were 3.7 ± 0.2, 2.7 ± 0.1, and 2.1 ± 0.1 min for CE, CH, and LI, respectively, (Figures 2, 3).

***Optimization of TLC-densitometric method***

*Optimization of developing system:* Different developing systems of different compositions were tried as methanol: chloroform, methanol: ethyl acetate, and methanol: acetone in different ratios. Ammonia and acetic acid solutions were added separately to the last system in different ratios where addition of acetic acid solution to make a system of methanol: acetone: acetic acid (7: 3: 0.2, by volume) removed tailing and improved spots shape and resulted in optimum separation with good peaks symmetry, (Figure 4, 5).

*Optimization of scanning wavelength:* Different scanning wavelengths were tried. The best scanning wavelength was 215 nm which showed good signal to noise ratio for all components resulting in high sensitivity.

*Optimization of slit dimensions of scanning light beam:* Different band dimensions were tested. The optimum band width chosen was 4 mm with 8.9 mm inter-space between bands. Different slit dimensions were tried, where 4 mm × 0.45 mm proved to be the slit dimensions of choice which provide high sensitivity.

Acceptable chromatographic separation for the ternary mixture was achieved upon using the above optimum conditions. The respective compounds were well separated at reasonable retention times, where the Rf values were 0.12 ± 0.01, 0.81 ± 0.02, and 0.48 ± 0.01 for CE, CH, and LI, respectively, (Figure 4, 5).

**Methods validation**

Methods validation was performed with respect to ICH guidelines **(52)**.

***Linearity***

Beer’s Lambert’s law was obeyed in the concentration ranges of 1-30, 0.5-30 and 1-50 μg mL-1 for CE, CH, and LI, respectively (for RP-HPLC method) and in the range of 0.1-5, 0.2-3 and 0.4-6 μg band-1 for CE, CH, and LI, respectively (for TLC-densitometric method). Regression parameters like correlation coefficients, intercept and slope values were calculated and presented in (Table I).

***Accuracy***

Accuracy was calculated as % recovery of pure CE, CH, and LI as shown in (Table I). Accuracy was further evaluated by application of standard addition technique by addition of known amounts of pure drugs, at different levels (50, 100, 150, and 200%), to known concentrations of Canyon® gel and then analyzing the prepared mixtures. Acceptable results were given in (Table II).

***Precision***

It was studied with respect to intra-day (repeatability) and inter-day (intermediate precision). Repeatability was evaluated by repeating the assay of three different concentrations three times in the same day while intermediate precision was evaluated by assaying the same samples in triplicates on three successive days, using the developed chromatographic methods and calculating the percentage recoveries and RSD % values. Results in (Table I) confirmed the satisfactory precision of the proposed methods.

***Limits of detection and quantitation***

Limits of detection and quantitation for CE, CH, and LI were shown in (Table I) which showed adequately small values indicating the high sensitivity of the proposed methods.

***Selectivity***

The selectivity of the methods was confirmed by good resolution of the proposed drugs as shown in the chromatograms (Figures 2, 4). Also good percentage recoveries obtained upon applying the methods to Canyon® gel proved the high selectivity of the proposed methods and that there was no interference from excipients, (Figures 3, 5) (Table II).

***Robustness***

Robustness of the methods was evaluated by calculating the percent relative standard deviation (%RSD) of peak areas for each studied parameter. It was established by making small deliberate changes in the chromatographic parameters, e.g. changing in flow rate by ± 0.1 mL min-1, changing in phosphoric acid, acetonitrile, and methanol ratio in the mobile phase by ±2, ±1, and ±3 %, respectively, changing the strength of phosphoric acid by ± 0.001 (for RP-HPLC method), changing methanol and acetone ratio of the developing system by ±1 and ±0.3%, changing acetic acid percent by ± 0.02 (for TLC-densitometric method) then calculating the resolution among the studied drugs. It was found that the changes in the studied parameters have no significant effect on the chromatographic resolution.

***System suitability testing parameters***

The system suitability test confirms that the analytical procedure is valid as well as ensures the resolution between different peaks of interest. System suitability testing was carried out during methods development and optimization according to ICH guidelines **(52)**. Resolution (Rs) and selectivity (α) factors were calculated and were found to be >2 and 1.5, respectively, for all drugs. In addition, the symmetry factors were calculated for the three drugs and nearly equaled 1. Other parameters such as capacity factor, number of theoretical plates and height equivalent to theoretical plates were calculated, and their values were within the acceptable limits (Table IV).

**Conclusion**

The developed chromatographic methods were the first developed methods for determination of CE, CH, and LI in their ternary mixtures in pure form with successful application to pharmaceutical formulations. The proposed RP-HPLC method had some advantages over the reported HPLC-DAD method, **(51)** that it provided separation for CE, CH, and LI in their ternary mixture only within 4 minutes using the simple isocratic elution. While the proposed TLC-densitometric method, had the high sensitivity required for determination of CE, CH, and LI even in the ratio of 1:5:50, pharmaceutical formulation ratio, with high precision, and selectivity. Also the proposed TLC-densitometric method was time saving that several samples could be run simultaneously using small quantity of the developing system. The aforementioned advantages made them preferable methods for quality control of the studied drugs. Moreover, all the obtained results confirmed the applicability, accuracy and precision of these methods.

**References**

[1] The British Pharmacopoeia, Her Majesty’s. The Stationary Office (2007) London, UK, Vol.I and II: Electronic version.

[2] The United States Pharmacopoeia (2007) National Formulary 25, United States Pharmacopoeia Convention Inc., 30thedn, 713: 1427.

[3] Asadoorian, Joanna, Williams, Karen; cetylpyridinium chloride mouth rinse on gingivitis and plaque; Journal of Dental Hygiene, (2008); 82 (5): 2-8.

[4] Haps, S., Slot, D. E., Berchier, C. E.; Weijden, G. A.; The effect of cetylpyridinium chloride-containing mouth rinses as adjuncts to tooth brushing on plaque and parameters of gingival inflammation; International Journal of Dental Hygiene, (2008); 6 (4): 290-303.

[5] Sweetman SC (34thEdn.), Martindale: The Complete Drug References. London, UK: Pharmaceutical Press. Electronic version, (2005)

[6] Sporkert, F., Pragst, F.; Determination of lidocaine in hair of drug fatalities by headspace solid-phase micro extraction; Journal of Analytical Toxicology, (2000); 24 (2): 316-322.

[7] Cox, S. K., Hamner, T., Bartges, J.; Monoethylglycinexylidide and lidocaine determination in porcine microsomal preparations; Journal of Pharmaceutical and Biomedical Analysis, (2005); 37 (5):801-804.

[8] Gamze, T., Gulcin, B., Mehmet, O. A., Serdar, A.; Determination of lidocaine based on electro catalysis of a chemically modified electrode; Turkish Journal of Chemistry, (2012); 36 (9): 593.

[9] Jong, G. J., Koster, E. H. M.; Solid-Phase micro-extraction in bioanalysis, exemplified by Lidocaine determination; Chromatographia, (2000); 52 (11): 512-518.

[10] Oliveria, R. T. S., Banda, G. R. S., Ferreia, V. S., Oliveria, S. C., Avaca, L. A.; Electroanalytical determination of Lidocaine in pharmaceutical preparations using Boron-Doped Diamond Electrodes; Electroanalysis, (2007); 19 (2): 1189-1194.

[11] Deglin, S. M., Deglin, J. M., Wurtzbacher, J., Litton, M., Rolfe, C., Carolyn, M. R. N.; Rapid serum lidocaine determination in the coronary care unit; The Journal of the American Medical Association, (1980); 244 (22): 571-573.

[12] Benamor, M., Aquersif, N., Draa, M. T.; Spectrophotometric determination of cetylpyridinium chloride in pharmaceutical products; Journal of Pharmaceutical and Biomedical Analysis, (2001); 26 (1): 151-154.

[13] Parham, H., Pourreza, N., Moradi, D.; Development of a flotation-spectrophotometric method for determination of cetylpyridinium chloride in pharmaceutical products; Quimica Nova, (2011); 34 (5): 1-4.

[14] Zarei, A. R., Sadeghi, H. B., Abedin, S.; Selective Cloud Point Extraction for the Spectrophotometric Determination of Cetylpyridinium Chloride in Pharmaceutical Formulations; Iranian Journal of Pharmaceutical Research, (2013); 12 (4): 671-677.

[15] Montes, L. H. C., Cassella, R. J.; Reversed Flow Injection System for the Spectrophotometric Determination of Cetylpyridinium Chloride in Pharmaceutical Products with Eriochrome Black T in Triton X-100 Medium; J. Flow Injection Anal., (2010); 27 (1): 42-48.

[16] Taylor, R. B., Toasaksiri, S., Reid, R. G., Wood, D.; Determination of the Quaternary Ammonium Compounds Dequalinium and Cetylpyridinium Chlorides in Candy-based Lozenges by High-performance Liquid Chromatography; Analyst, (1997); 122 (9): 973-976.

[17] Meesri, J., Kongthong, S.; Method Validation of Cetylpyridinium Chloride Determination in Mouthwash Products by HPLC; Bulletin of the Department Medical of Sciences, (2002); 44 (2): 120-124.

[18] Wang, J., Lu, J., Zhang, L., Hu, Y.; Determination of cetylpyridinium chloride and tetracaine hydrochloride in buccal tablets by RP-HPLC Journal of Pharmaceutical and Biomedical Analysis, (2003); 32 (2): 381-386.

[19] Morales, S. R., Zhou, X., Salari, H., Castillo, R., Breen, P., Compadre, C. M.; Liquid chromatography determination of residue levels on apples treated with cetylpyridinium chloride; Journal of Chromatography A, (2005); 1062 (4): 285-289.

[20] Nakov, N., Acevaska, J., Brezovska, K., Petkovska, R., Dimitrovska, A.; Optimization of hydrophilic interaction liquid chromatographic method for simultaneous determination of cetylpyridinium chloride and benzocaine in lozenges; Macedonian Journal of Chemistry and Chemical Engineering, (2012); 31 (1): 35-38.

[21] Morioka, H., Nozaki, Y., Kabayama, K., Misawa, N.; Determination of cetylpyridinium chloride residue in chicken meat by hydrophilic interaction chromatography; Journal of Liquid Chromatography and Related Technologies, (2014); 37 (4): 538-547.

[22] Wyhowski De Bukanski, B.; Analysis of domiphen bromide and cetylpyridinium chloride in cosmetic products by high-performance liquid chromatography; International Journal of Cosmetic Science, (2007); 9 (4): 193-198.

[23] Šimůnková-Kulová, P., Kotouček, M.; Indirect Voltammetric Determination of Cetylpyridinium Chloride Using Ion Pair Extraction; Acta Universitatis Palackianae Olomucensis, (2003); 42 (1): 51-60.

[24] Mohamed, G. G., Ali, T. A., El-Shahat, M. F. Sabagh, A. M., Migahed, M. A., Khaled, E.; Potentiometric determination of cetylpyridinium chloride using a new type of screen-printed ion selective electrodes; Analytica Chimica Acta, (2010); 673 (1): 79-87.

[25] Mostafa, G. A.; PVC Matrix Membrane Sensor for Potentiometric Determination of Cetylpyridinium Chloride; Analytical Sciences, (2001); 17 (9): 1043-1047.

[26] Campanella, L., Sammartino, M. P., Sbrilli, R., Tomassetti, M.; Analytical comparison of an enzyme-amperometric method for chlorocresol determination in ointments with colorimetry and liquid chromatography; Journal of Pharmaceutical and Biomedical Analysis, (1992); 10 (10-12): 751-755.

[27] Bloomfield, M. S., Prebble, K. A.; The determination of the preservative, chlorocresol, in a pharmaceutical formulation by flow injection analysis; Journal of Pharmaceutical and Biomedical Analysis, (1992); 10 (10-12): 775-778.

[28] Gatti, R, Roveri, P., Bonazz, D., Cavrini, V.; HPLC-fluorescence determination of chlorocresol and chloroxylenol in pharmaceuticals; Journal of Pharmaceutical and Biomedical Analysis, (1997); 16 (3): 405-412.

[29] Shaikh, S., Muneera, M. S., Thusleem, O. A., Tahir, M., Anand, V. K.; A Simple RP-HPLC Method for the Simultaneous Quantitation of Chlorocresol, Mometasone Furoate, and Fusidic Acid in Creams; Journal of Chromatographic Science, (2009); 47 (3): 178-182.

[30] Lee, J. G., Shin, S. Y., Shin, H. J., Huh, Y., Seul, J. L., Kim, D. H., Lee, S, Kim, Y. O.; Determination of three preservatives, cresol, chlorocresol and benzethonium, in drugs by high performance liquid chromatography–ultraviolet (HPLC–UV) detection; Journal of Pharmaceutical Investigation, (2012); 42 (1): 47-50.

[31] Johnston, S.E., Gill, N.L., Wei, Y.-C., Markovich, R., Rustum, A.M.; Development and validation of a stability-indicating RP-HPLC method for simultaneous assay of betamethasone dipropionate, chlorocresol, and for the estimation of betamethasone dipropionate related compounds in a pharmaceutical cream and ointment; Journal of Chromatographic Science, (2010); 48 (9): 733-741.

[32] Turabi, Z. M., Khatatbeh, O. A.; Simultaneous Determination of Clobetasol (as Propionate) and Chlorocresol in Cream by Stability Indicating RP-HPLC Method; International Journal of Pharmaceutical Sciences and Drug Research, (2014); 6 (2): 140-144.

[33] Lakka, N. S., Goswami, N.; A Stability Indicating RP-HPLC Method For Simultaneous Determination of Halobetasol Propionate and P-Chlorocresol in Creams; International Research Journal of Pharmaceutical and Applied Sciences, (2012); 2 (5): 13-19.

[34] Zang, L., Barbero, A. M., Frasch, H.F.; A Rapid HPLC Analysis for Dermal Penetration: The Case Study of 4-Chloro-3-Methylphenol (CMP) from Metal Working Fluid Trim VX; The Open Analytical Chemistry Journal, (2010); 4 (1): 10-17.

[35] Abdelwahab, N. S., Nouruddin, W. A., EL Fatatry, M. H., Osman, W. M.; Determination of Thiomersal, Lidocaine and Phenylephrine in their Ternary Mixture; Journal of Chromatography and Separation Techniques, (2013); 4 (8): 1-6.

[36] Edward, G., Feldmann, Henry, M.; The colorimetric determination of lidocaine with cis-aconitic anhydride; Journal of the American Pharmaceutical Association, (1959); 48 (10): 549-552.

[37] Wiberg, K., Hagman, A., Jacobsson, S. P.; Rapid determination of lidocaine solutions with non-column chromatographic diode array UV spectroscopy and multivariate calibration; Journal of Pharmaceutical and Biomedical Analysis (2003); 30 (5): 1575-1586.

[38] Plenis, A., Konieezna, L., Miekus, N., Baczek, T.; Development of the HPLC Method for Simultaneous Determination of Lidocaine Hydrochloride and Tribenoside Along with Their Impurities Supported by the QSRR Approach; Chromatographia, (2013); 76 (5-6): 255-265.

[39] Chik, Z., Lee, T. D., Holt, D. W., Johnston, A., Tucker, A. T.; Validation of High-Performance Liquid Chromatographic–Mass Spectrometric Method for the Analysis of Lidocaine in Human Plasma; Journal of Chromatographic Science, (2006); 44 (5): 260-265.

[40] Júnior, E. R., Bentley, M. V. L., Marchetti, J. M.; HPLC assay of lidocaine in vitro dissolution test of the Poloxamer 407 gels; Brazilian Journal of Pharmaceutical Sciences, (2002); 38 (1): 107-111.

[41] Helboe, P., Thomsen, M.; Single dose determination of suppositories containing phenylephrine, Lidocaine and beta methasone valerate by reversed-phase ion pair liquid chromatography; International Journal of Pharmacy, (1979); 2 (6): 317-324.

[42] Kang, L., Jun, H. W., McCall, J. W.; HPLC assay of Lidocaine in plasma with solid phase extraction and UV detection; Journal of Pharmaceutical and Biomedical Analysis, (1999); 19 (22): 737-745.

[43] O'Neal, C. L., Poklis, A.; Sensitive HPLC for simultaneous quantification of lidocaine and its metabolites monoethylglycinexylidide and glycinexylidide in serum; Clinical Chemistry, (1996); 42 (4): 330-331.

[44] Zargar, B., Hatamie, A.; Hollow fiber liquid based microextraction combined with high-performance liquid-chromatography for the analysis of lidocaine in biological and pharmaceutical samples; Analytical Methods, (2014); 6 (8): 2506-2511.

[45] He, Y.T., Peng, J.D., Tang, J.X., Zhang, C.; Incorporation of high performance liquid chromatography with resonance Rayleigh scattering detection for determination of procaine and lidocaine in human plasma; Analytical Methods, (2013); 5 (24): 7110-7116.

[46] Belal, T.S., Haggag, R.S.; Gradient HPLC-DAD stability indicating determination of miconazole nitrate and lidocaine hydrochloride in their combined oral gel dosage form; Journal of Chromatographic Science, (2012); 50 (5): 401-409.

[47] Pendela, M., Kahsay, G., Baekelandt, I., Van Schepdael, A., Adams, E.; Simultaneous determination of lidocaine hydrochloride, hydrocortisone and nystatin in a pharmaceutical preparation by RP-LC; Journal of Pharmaceutical and Biomedical Analysis, (2011); 56 (3): 641-644.

[48] Shaalan, R.A., Belal, T.S.; HPLC-DAD stability indicating determination of nitrofurazone and lidocaine hydrochloride in their combined topical dosage form; Journal of Chromatographic Science, (2010); 48 (8): 647-653.

[49] Qin, W.-w., Jiao, Z., Zhong, M.-k., Shi, X.-j., Zhang, J., Li, Z.-d., Cui, X.-y.; Simultaneous determination of procaine, lidocaine, ropivacaine, tetracaine and bupivacaine in human plasma by high-performance liquid chromatography; Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, (2010); 878 (15-16): 1185-1189.

[50] Jancic-Stojanović, B., Malenović, A., Marković, S., Ivanović, D., Medenica, M.; Optimization and validation of an RP-HPLC method for analysis of hydrocortisone acetate and lidocaine in suppositories; Journal of AOAC International, (2010); 93 (1): 102-107.

[51] Belal, T.S., Shaalan, R.A., Haggag, R.S.; Gradient HPLC-diode array detector stability-indicating determination of lidocaine hydrochloride and cetylpyridinium chloride in two combined oral gel dosage forms; Journal of AOAC International, (2011); 94 (2): 503-512.

[52] ICH, Q2 (R1) Validation of Analytical Procedures, Proceedings of the International Conference on Harmonization, Geneva, 2005.

[53] Harvey, D., Analytical Chemistry 2.0, Chapter 5: Standardizing Analytical Methods, pp. 160,SourceURL:<http://www.asdlib.org/onlineArticles/ecourseware/Analytical%20Chemistry%202.0/Text_Files.html>Saylor URL: http://www.saylor.org/courses/chem108

**Figure captions**

**Figure 1** Chemical structure of cetylpyridinium chloride (a), chlorocresol (b), and Lidocaine (c).

**Figure 2** HPLC chromatogram of 2 µg mL-1each of cetylpyridinium chloride (CE), chlorocresol (CH), and lidocaine (LI) using 0.05% phosphoric acid solution: acetonitrile: methanol (15: 24: 61, by volume) as a mobile phase.

**Figure 3** HPLC chromatogram of Canyon® gel sample containing 1, 5, 50 µg mL-1 each of cetylpyridinium chloride (CE), chlorocresol (CH), and lidocaine (LI), respectively using 0.05% phosphoric acid solution: acetonitrile: methanol (15: 24: 61, by volume) as a mobile phase.

**Figure 4** TLC-densitogram of a mixture containing 0.1 µg band-1 cetylpyridinium chloride (CE), 0.5 µg band-1 chlorocresol (CH), and 5 µg band-1 lidocaine (LI) using methanol: acetone: acetic acid (7: 3: 0.2, by volume) as a developing system.

**Figure 5** TLC-densitogram of Canyon® gel sample containing 0.1, 0.5, and 5 µg band-1 cetylpyridinium chloride (CE), chlorocresol (CH), and lidocaine (LI), respectively using methanol: acetone: acetic acid (7: 3: 0.2, by volume) as a developing system.