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SIMULTANEOUS DETERMINATION OF PROCAINE HYDROCHLORIDE, PROCAINAMIDE HYDROCHLORIDE AND LIDOCAINE BY MOLECULAR ABSORPTION SPECTROMETRY

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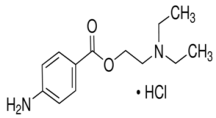
Abstract
Two spectrometric methods have been developed for quantitative simultaneous determination of procaine hydrochloride (PH·HCl), procainamide hydrochloride (PHA·HCl) and lidocaine (Lid) from synthetic mixture. The methods employed are first derivative spectrometry, using zero crossing method and multicomponent analysis which is based on the additivity law. Using first derivative spectrometry, the wavelength selected for the quantitative determination of PH·HCl was 237 nm for Lid was 242 nm and for PHA·HCl was 290 nm in mixture. The method is linear when the concentration ranged between 6.62-9.93 µg/mL for PH·HCl, 6.43-9.64 for PHA·HCl and 5.56-8.35 for Lid. The multicomponent analysis is a direct method and involves the absorbance measurements of at three different wavelengths. The molar absorption coefficients values were calculated at each wavelength and the concentration of PH·HCl, PHA·HCl and Lid from mixture was determined by solving matrix using Cramer's rule. The recovery of each compound in mixture was calculated and it is 101.4 % for PH·HCl, 100.4 % for PHA·HCl and 98.4 % for Lid.

Keywords*: first derivative spectrometry, multicomponent analysis, procaine hydrochloride, procainamide hydrochloride, lidocaine*

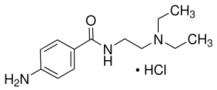
Introduction

Procaine hydrochloride (PH·HCl), procainamide hydrochloride **(**PHA·HCl) and lidocaine (Lid) are chemicals compounds with local anesthetics properties. Those compounds can be divided into two groups: the amino-amides (PHA·HCl) and amino-esters (Lid and PH·HCl). The PH·HCl (2-diethyaminoethyl-4-amino benzoate hydrochloride) is the active substance in Romanian medical products Gerovital H_3 and Aslavital, used in anti-aging treatments. PHA·HCl (4-amino-N-(2-diethylaminoethyl) benzamide hydrochloride) is an antiarrhythmic drug used to treat ventricular arrhythmias. Lidocaine (4-amino-N-(2-diethylaminoethyl) benzamide) is a local anesthetic widely used for major pain during epidural, peripheral and central spinal anesthesia (Qin et al. 2010; Quiroz et al. 2019; Kumar et al. 2012). In spite of the fact that numerous methods, such as UV-VIS (Tamarh et al. 2012; Kumar et al. 2012), RP- HPLC (Qin et al. 2010; Lian et al. 2019; Jadac et al. 2012) and LC-MS/MS (Manna et al. 2019; Lian et al. 2019) have been reported to determine these compounds. The aim of this article was to develop two simple, rapid, accurate and precise methods for simultaneous quantitative determination of PH·HCl, PHA·HCl

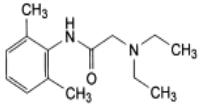
and Lid from the synthetic mixture. The chemical structure of those compose are presented in Figure 1.



Procaine hydrochloride



Procainamide hydrochloride



Lidocaine

Figure 1. Structures of PH·HCl, PHA·HCl and Lid

Materials and Methods

Chemicals

All chemicals and solvents used in this study have the analytical grades. The PH·HCl (99%), PHA·HCl (99%) and Lid (97.5%) were purchased from Across Organics Company and the methanol was obtained from Sigma-Aldrich.

Apparatus

The measurements were carried out using a Jasco V 530 double beam spectrometer coupled to a PC computer, running the Jasco software. All spectra measurements have been made in quartz cells of 1 cm path-length. The spectra were recording for wavelengths in range of 200-400 nm at a scanning speed of 100 $nm \cdot min^{-1}$. Microsoft office excel 2007 was used for linear regression analysis.

Stock solution and working solutions

The *stock solutions* of 1 mM were obtained by dissolving the suitable quantity of procaine hydrochloride, procainamide hydrochloride or lidocaine in 5 mL methanol then filled up to 25 mL in volumetric flask mark with distilled water.

Working solutions The 0.04 mM working solution were prepared from a volume of 1mL stock solution of PH·HCl, PHA·HCl or Lid in 25 mL volumetric flask. For validation method have been prepared different solution by diluting the stock solution in distilled water.

Mixture solution A volume of 1 mL from 1mM stock solution of each compound was quantitatively transferred in 25 mL volumetric flask and filled up to mark with distilled water.

Procedures

The UV spectra of all the solutions are recorded in the range of 200-400 nm. The zero order spectra of each solution was detected against a blank solution which did not contain the studied compounds. First derivative spectrum has been obtained using the Jasco software.

Results and Discussion

The quantitative determination of organic compounds with whose structure ensures the existence of UV-VIS spectrum is easy to measure if is only one species in the mixture. In addition, if the sample to be analyzed contains several organic compounds and whose spectra is overlaped, depending on their structure, specific method called derived spectrometry (Badea et al. 2002; Badea & Vladescu 2015;

Moldovan & Alexandrescu 2004; Zimmer & Czarnecki 2010) or multicomponent analysis can be developed to detected of them.
In our experiment the absorption spectra of PH·HCl (11.04 µg/mL), PHA·HCl (10.72 µg/mL) and Lid (9.28 µg/mL) as well as their mixture (11.04 - 10.72 - 9.28 µg/mL) showed a high degree of overlapping in the range of 200-350 nm which made impossible to simultaneously determinate the PH·HCl, PHA·HCl and Lid (Figure 2).
This issue of quantification the PH·HCl, PHA·HCl and Lid was solved by using the first derivative spectrometry (Figure 3). The results showed that at 237 and 279 nm, 242 and 290 nm or 258 and 284 nm the derivative signals of the PHA·HCl, PH·HCl and Lid passed through zero. Therefore, based on zero crossing first derivative spectrometry principle at 242 nm, 290 nm and 237 nm were selected the optimum working wavelengths for the quantitative determination of Lid, PHA·HCl and PH·HCl from the mixture without the need of prior separation before analysis.

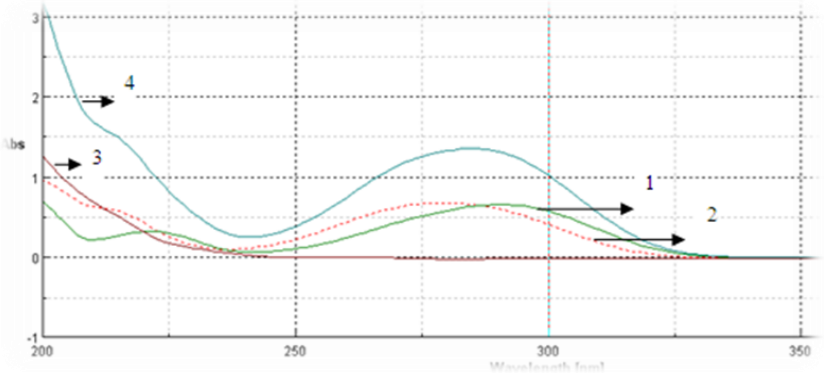


Figure 2. Absorption spectra of: 1. PH·HCl (11.04 µg/mL), 2. PHA·HCl (10.72 µg/mL), 3. Lid (9.28 µg/mL) and 4. mixture of PH·HCl, PHA·HCl and Lid (11.04 - 10.72 - 9.28 µg/mL)

Validation of the derivative method
The first derivative method developed in this study was validated in accordance with ICH guideline. Therefore, the validation steps were linearity, limits of detection and quantification, accuracy and precision (Badea et al. 2012, Cruceru et al. 2011, ICH Q2(R1) 2015).
The linearity of method was verified on five working solutions prepared for every compound. The concentrations of working solutions were ranged from 6.62-9.93 µg/mL for PH·HCl, 6.43-9.64 µg/mL for PHA·HCl and 5.56-8.35 µg/mL for Lid.
The parameters of linearity showed that the intercept values are small and the correlation coefficient is close to one for all the studied compounds (Table 1) which suggests can the Lambert Beer law is verified.

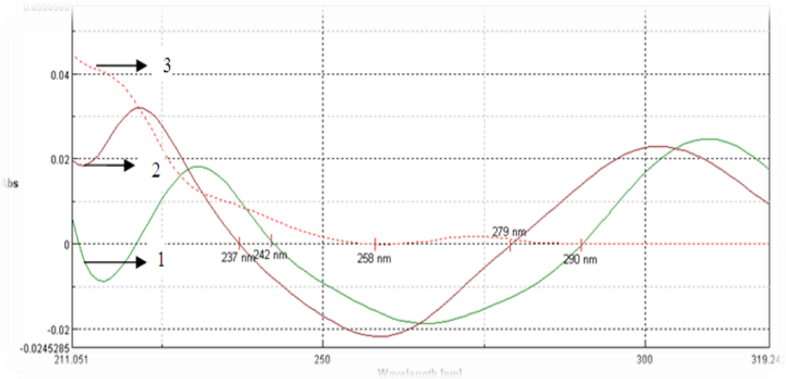


Figure 3. First derivative spectra of 1. PH·HCl (11.04 µg/mL), 2. PHA·HCl (10.72 µg/mL), 3. Lid (9.28 µg/mL)

Table 1. Results obtained for linearity, LOD and LOQ studies

Compound	Linearity range (µg/mL)	Regression equations	R^2	LOD µg/mL	LOQ µg/mL	Λ nm
PH·HCl	6.62-9.93	y=0.0018x + 0.0004	0.9975	0.01	0.06	231
PHA·HCl	6.43-9.64	y=0.0020x + 0.0015	0.9961	0.01	0.04	302
Lid	5.56-8.35	y=0.0002x + 0.0001	0.9955	0.07	2.78	274

Detection and quantification limits were established as 3:1 and 10:1 signal to noise ratio. The LODs and LOQs determined and are presented in Table 1.
Accuracy has been determined by calculating the recovery of PH·HCl, PHA·HCl and Lid from stock solutions. For this purpose different solutions have been prepared in triplicate at three levels of concentration, covering the range 80%, 100% and 120%. Results determined from experimental studies are shown in Table 2. Retrieval yields were ranged between 99.4 and 101% results that corresponds to the criterion for this validation parameter.
The precision was estimated by repeatability and intermediate precision which is evaluated by determination of the relative standard deviation (RSD, %). The results are presented in Table 3. The RSD values calculated are less than 2% for each compound.

Table 2. Results obtained for accuracy

Compound	Conc. range (µg/mL)	Added (µg/mL)	Found[1] (µg/mL)	Recovery[1] [% ± sd]	Average recovery[1]	RSD %
PH·HCl	80%	6.62	6.675±0.008	100.8±0.115		
	100%	8.28	8.311±0.007	100.4±0.083	100.8	0.384
	120%	9.93	10.049±0.015	101.1±0.148		
PHA·HCl	80%	6.43	6.568±0.011	102±0.017		
	100%	8.04	7.942±0.038	98.8±0.474	100.5	1.619
	120%	9.64	9.722±0.010	101±0.107		
Lid	80%	5.56	5.504±0.016	99±0.291		
	100%	6.96	6.891±0.030	99±0.428	99.4	0.706
	120%	8.35	8.368±0.045	100.2±0.541		

[1]each result represents mean calculated for three determinations ± standard deviation

Table 3. Values of precision calculated

Compound	Added ($\mu g \cdot mL^{-1}$)	Found intraday [1] ($\mu g \cdot mL^{-1}$)	Found interday [1] ($\mu g \cdot mL^{-1}$)	RSD% Intraday	RSD% Interday (after one day)
PH·HCl	8.28	8.366	8.333	0.28	0.29
PHA·HCl	8.04	8.001	8.005	0.14	0.26
Lid	6.96	6.932	6.955	0.74	0.66

[1]each result represents mean calculated for six determinations

Multicomponent analysis using molecular absorption spectrometry in visible range
The present paper used the performances of multicomponent analysis can involve to solving *n* equations and *n* unknown systems, but in the same time the choosing adequate of wavelengths to determined accurate experimental results (Samah et al 2016).
Development of the multicomponent method in this study, the additivity law is employed for quantitative simultaneous determination of PH·HCl, PHA·HCl and Lid in the mixture. In order to calculate the concentration of these compounds, a mathematical system equal to the number of analytes was employed, and in consequence we have three equations. The matrix methodology was used to solve the system of equations with three unknowns and Cramer's rule have been involved to the matrix developed. The result obtained represents the concentration of PH·HCl, PHA·HCl and Lid from the mixture. The absorption spectra of these compounds and their mixture were presented in Figure 1. The measurement of absorbances was carried out at three different wavelengths of 222 nm (λmax of PH·HCl), 217 nm (λmax of PAH·HCl) and 216 nm (λmax of Lid) and the molar absorption coefficient values were calculated. The found concentrations of PH·HCl, PHA·HCl and Lid obtained by solving the equations system is illustrated in Table 4. Moreover, the recovery of each compound was calculated and the results detected were presented in Table 4.

Table 4. Results obtained when the multicomponent method was applied on the mixture sample

Compound	Added ($\mu g \cdot mL^{-1}$)	Found ($\mu g \cdot mL^{-1}$)	Recovery %
PH·HCl	11.31	11.53	101.4
PHA·HCl	11.36	11.41	100.4
Lid	9.28	9.13	98.4

Conclusions

The first derivative spectrometry and multicomponent analysis methods could be used with very good results for quantitative simultaneous determination of PH·HCl, PHA·HCl and Lid from synthetic mixture. The first derivative method was validated by checking the linearity, limit of detection and limit of quantification, accuracy and precision. The multicomponent method was found to be simple, sensitive, accurate and represents a better alternative to the other methods for the determination of PH·HCl, PHA·HCl and Lid from synthetic mixture.

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