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Spectrophotometric determination of lithium with Quinizarin in drugs and serum

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Abstract

A very sensitive analytical method is proposed for the determination of lithium based on the reaction of Li^+ ion wiht 1,4-dihydroxyanthraquinone (Quinizarin). In dimethylsulfoxide medium (90%) and in the presence of sodium hydroxide and sodium carbonate, a bluish-violet color ($\lambda_{max} = 601$ nm) develops and is stable over a period of 30 min to 2.5 h. The NaOH and Quinizarin concentrations were optimized simultaneously using the response surface methodology from sequential experimental Doehlert designs. Beer's law is obeyed in the concentration range 14–250 ppb Li^+ in aqueous and serum matrices, and the errors (RSD) in the determination of 100 ppb Li^+ are 4.0% and 3.9% respectively. The proposed procedure was satisfactorily applied to the determination of lithium in drugs and human serum (no deproteinization is required).

Keywords: Doehlert experimental design; Lithium; Quinizarin; Visible-spectrophotometry

1. Introduction

Lithium, like other alkali metals, shows relatively poor chemical coordination. However, its high charge density provides greater affinity to ligands with donor oxygen atoms than the rest of the alkali metals and therefore lithium forms stable chelates in solution more easily [1,2]. Therefore, few organic reagents which form colored chelates with alkali metals are known.

Several chromogenic organic reagents with two aromatic rings linked by an azo group (–N=N–) with ortho substituents –OH in one ring and –COOH, $-PO(OH)_2$ or $-AsO(OH)_2$ in the second ring have been used as complexing agents of Li^+ (Thoron, Quinazolinazo, Nitroantranylazo, Phosphonazo R, Arsenazo III) in photometric determinations [3]. Among these, Thoron (*o*-(2-hydroxy-3,6-disulfo-1-naphthylazo)benzenearsonic acid) has been the most used. Proposed by Kuznetsov [4], it gives, together with Li^+ ion, an orange color in a strong basic medium. The spectrophotometric method using this reagent, developed by Thomason [5], has been used for the determination of lithium in high-purity beryllium and beryllium oxide [6], sea water [7], mollusc shells [8] and blood serum [9].

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Multidentate ligands derived from 3-fenilformazano ($NH{=}N{-}C(Ph){=}N{-}NH_2$) have also been suggested as spectrophotometric reagents for Li^+ [10–14].

Recently, with the development of macrocyclic ligands such as crown and aza-crown ethers, cryptands and spherands, new spectrophotometric methods for the determination of lithium in drugs, serum and urine samples have been proposed. These methods show good selectivity against other alkali metals but due to the low solubility of this type of ligand anhydrous media [15], extraction processes [16,17] or the use of a water–micellar medium is required for solubilization [18].

Quinizarin (1,4-dihydroxyanthraquinone) is a chromogenic and fluorogenic agent of metallic ions that shows high selectivity in its reactions since the O-donors are blocked by two strong intramolecular hydrogen bondings [19]; therefore, few spectrophotometric methods using this reagent are known. This reagent has been applied for the determination of Mg^{2+} [20] and UO_2^{2+} [21] and for the simultaneous determination of Lu^{3+} and Pr^{3+} [22] and Tm and Nd [23]. Other similar reagents have also been used occasionally such as Quinizarin-sulfonic acid (1,4-dihydroxy-anthra quinone-2-sulfonic acid) for Be^{2+} [24] and Al^+ [25]; Leucoquinizarin(1,4,9,10-tetrahydroxyanthracene) for Mg^{2+} [26] and Be^{2+} [27], and naphthazarin (*5,8-dihydroxy-1,4-naphthoquinone*) for Th^{4+} [28], and Th^{4+} and UO_2^{2+} simultaneously [29].

Response surface methodology (RSM) [30] is a group of mathematical and statistical techniques used for analyzing and modeling a problem where a particular response is a function of several variables, and where the aim is to optimize this response. RSM obtains an appropriate estimate of the real functional relationship between the instrumental response and the experimental factors under study. A quadratic function is the best solution as it is a simple model which can describe a great variety of surfaces [31], allows the prediction of the existence of curvature in the system and permits the computation of the maximum coordinates. In addition, this type of function can be simply obtained with a three-level experimental design.

In this paper, a sensitive method for the spectrophotometric determination of lithium in dimethylsulfoxide: water medium in the range 14–250 ppb lithium is proposed. The method is suitable for the clinical assay of lithium.

2. Experimental

2.1. Instrument

A Perkin-Elmer Lambda 5 UV–Vis spectrophotometer with two matched 1 cm quartz cells, thermostatically controlled at 25.0 ± 0.5°C with a water-bath circulator (Frigiterm S-382, J.P. Selecta), and a Corning 410 Flame Photometer were used.

A Casio FX-850 P pocket micro-computer with scientific library was used to calculate *P* values os statistic tests. All the calculations for optimization were carried out using the STATGRAPHICS data analysis package [32].

2.2. Reagents and materials

All materials, solvents and reagents were of analytical grade and were used without further purification. Doubly-distilled water was used throughout.

2.2.1. Lithium solution

A stock solution of Li^+ (500 ppm) was prepared by dissolving 1.24 g of lithium nitrate (Merck) in 250 ml of water. Working solutions were prepared by appropriate dilution of the stock solution.

2.2.2. Quinizarin solution

A 10^{-3} M solution was prepared by dissolving 60 mg of 1,4-dihydroxyanthaquinone (Merck) in 250 ml of dimethylsulfoxide.

2.2.3. Sodium hydroxide solution

A 1 M stock solution was prepared by dissolving 10 g of NaOH (Merck) in water. Working solutions were prepared daily by appropriate dilution of the stock solution.

2.2.4. Sodium carbonate solution

A 2.5 M stock solution was prepared by dissolving 66.237 g of sodium carbonate (Merck) in 250 ml of water. Working solutions were prepared by appropriate dilution.

2.3. Drug and serum

In order to verify the applicability of the proposed analytical method, the determination of lithium in the following real samples was validated

2.3.1. Otogén (Rimafar Laboratory S.A., Madrid, Spain)

Tablet containing lithium carbonate, potassium bromide, potassium iodide, thiamine and excipient. Mean weight of tablet: 319 mg.

2.3.2. Plenun (Lasa Laboratory, Barcelona, Spain)

Tablet containing lithium carbonate and exicipient. Mean weight of tablet: 516 mg.

2.3.3. Glucosor-Litio (Soria Natural, S.A., Soira, Spain)

Solution containing lithium gluconate, glucose and distilled water.

2.3.4. Serum

Blood serum from healthy individuals who had not been treated with lithium salts was centrifuged for 5 min at 4000 rev min^{-1} [33] and the supernatant liquid was separated and kept frozen at −15°C until the analysis. Once thawed, analyis must be done within 7 days [34]. Serum samples required no deproteinization.

2.4. General procedure for drugs

Using a micropipet, the indicated amount of standard or sample preparation was added to a test tube, followed by 50 μl of 0.1 M NaOH, 40 μl of 0.25 M Na_2CO_3 and water to make up to 1 ml. 250 μl of this solution was pipeted into another test tube, followed by 2.15 ml of dimethylsulfoxide and 100 μl of 10^{-3} M Quinizarin. The tubes were kept at 25°C in a thermostatic bath for 30 min. The absorbance of the solution was measured at 601 nm against a reagent blank prepared similarly. The calibration was made under identical conditions.

2.4.1. Otogen

Exactly 319 mg, obtained from 10 tablets previously powdered, was placed in a 100 ml glass beaker. 10 ml of 1 M hydrochloric acid was added and the solution was digested close to dryness. The residue was extracted with 10 ml of hot water, filtered, washed, transferred to a 100 ml volumetric flask and made up to volume with water. 40 μl of this solution was used for the analysis of lithium as described in section 2.4.

2.4.2. Plenur

The same procedure was followed, starting with 516 mg of the sample, transferring the final filtrate into a 250 ml volumetric flask and making up to volume with water. This solution was diluted 1:10 with water taking 50 μl for the subsequent analysis as described in section 2.4.

2.4.3. Glucosor-Litio

1 ml of sample was pipeted into a 10 ml volumetric flask and made up to volume with water. 100 μl of this solution was used, as described in section 2.4.

2.5. Procedure for serum

Accurately pipeted amounts (40, 50 and 70 μl) of standard lithium solution (50 ppm) were added to 500 μl of centrifuged serum and these solutions were diluted with water to make up to 1 ml. 100 μl portions of each of these spiked serum soltutions were pipeted into a test tube, followed by 100 μl of 0.1 M NaOH, 10 μl of 0.25 M Na_2CO_3, 40 μl of water, 2.15 ml of dimethylsulfoxide and 100 μl of 10^{-3} M Quinizarin. The procedure was completed as described in section 2.4. The calibration was made with lithium standards of increasing concentration, containing 100 μl of centrifuged pooled serum (obtained by mixing serum of 10 individuals) diluted 1:1 with water, and operating under the conditions described above.

3. Results and discussion

All the abosorbance measurements for optimization, calibration and interference experiments were performed against a corresponding solvent blank prepared under identical conditions.

3.1. Effect of dimethylsulfoxide: water ratio

The maximum absorption wavelengths and the absorbance difference between chelate and reagent are strongly dependent on the solvent. All the described spectrophotometric methods require a polar, water-miscible solvent and so water, methanol, ethanol, ethyline glycol, 2-propanol, acetone, acetonitrile, *N,N*-dimethylformamide, hexamethylphosphotriamide, dimethylsulfoxide, 1,4-dioxan and pyridine were tested in order to find the maximum absorbance difference. The results of this experiment are shown in Table 1. Dimethylsulfoxide was selected because it showed the maximum difference and the maximum hipsochromic displacement between the two spectra.

Table 1
Experimental values of wavelengths and absorbance for the system lithium–Quinizarin in different solvent/water mixtures (9/1, v/v) (Experimental conditions: $[Li^+] = 1.2 \times 10^{-4}$ M; $[NaOH] = 5 \times 10^{-3}$ M; $[Quinizarin] = 4.8 \times 10^{-5}$ M)

Solvent	λ_b[a]	λ_b[c]	$\Delta\lambda$[c]	$A^d{}_{max}(\lambda_d)$[e]
Water	550	550	0	0.023
Methanol	552	548	4	0.040
Ethanol	548	548	0	0.014
Ethylene glycol	547	547	0	0.040
2-Propanol	596	596	0	0.150
Acetone	600	595	5	0.294
Acetonitrile	595	590	5	0.186
DMF[f]	612	602	10	0.278
HMPT[g]	569	566	3	0.032
DMS[h]	616	604	12	0.294
1,4-Dioxan	604	596	8	0.286
Pyridine	570	565	5	0.232

[a] Maximum wavelength of the blank (nm).
[b] Maximum wavelenth of the chelate (nm).
[c] Difference betweeen [a] and [b] (nm).
[d] Maximum absorbance difference between chelate and reagent.
[e] Wavelength corresponding to maximum of [d] (nm).
[f] *N,N*-Dimethylformamide.
[g] Hexamethylphosphotriamide.
[g] Dimethylsulfoxide.

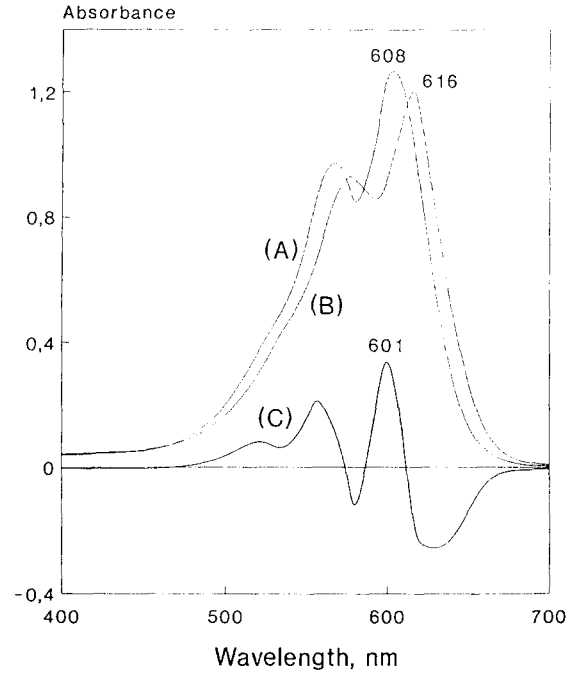

Fig. 1. Absorption spectra in 90% dimethylsulfoxide: (A) chelate; (B) ligand blank; (C) difference. ($[Li^+] = 1.2 \times 10^{-3}$ M, $[Quinizarin] = 4.8 \times 10^{-4}$ M, $[NaOH] = 5 \times 10^{-3}$ M)

As the proportion of water was increased, the absorbance of the chelate, measured against a reagent blank, decreased. Thus a proportion of 10% of water was selected as this provided sufficient aqueous phase for the preparation of the samples.

3.2. Absorption spectra

Fig. 1 shows absorption spectra of chelate and ligand blank against the solvent in 90% dimethylsulfoxide. Both curves show a maximum difference at 601 nm.

3.3. Effects of the Quinizarin and sodium hydroxide concentrations

Sodium hydroxide was selected (instead of KaOH or an organic base) in order to give an homogeneous background for the serum analysis. Molecular spectroscopic methods for the determination of lithium show a great interfering effect

from the other ions in solution, of which Na^+ is the principal one in a serum matrix, so in order to prevent possible interference from Na^+, NaOH was selected.

The NaOH and Quinizarin concentrations were optimized using the RSM from sequential experimental Doehlert designs [35] (Fig. 2), proposed by Bosque Sendra et al. [36,37], in order to obtain the maximum absorbance while simultaneously varying these two parameters for a constant lithium concentration of 1.2×10^{-4} M. These designs have never been used in optimization of experimental variables in analytical methods by molecular absorption spectrophotometry in solution.

As the central point, the values $-\log[\text{NaOH}] = 2.0$ and $10^5 \times [\text{Quinizarin}] = 2.0$ M were chosen for the first experimental design. Table 2 (Design I) shows the proposed Doehlert design and the experimental results obtained for the chelate absorbance measured against a reagent blank. Since

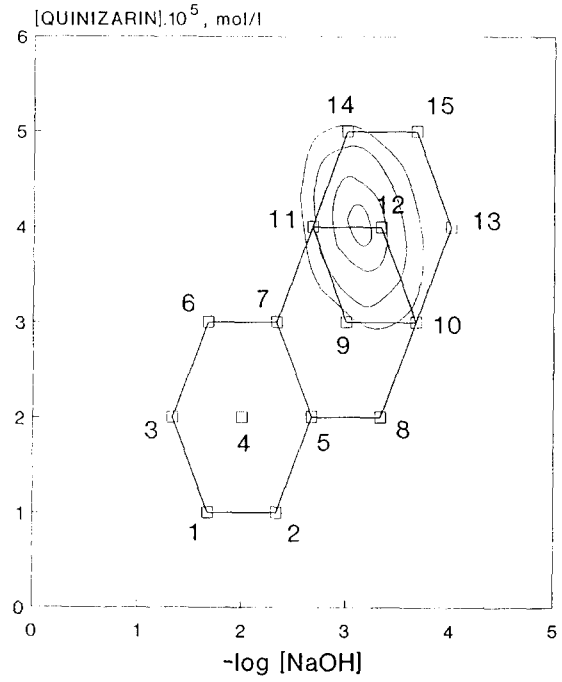

Fig. 2. Representation of the Doehlert designs I, II and III used for the simultaneous optimization of the Quinizarin and NaOH concentrations. Contour diagram of the response surface obtained from design III (the curves indicate 0.4, 0.5, 0.6 and 0.64 units of absorbance).

one of the factors is at five levels and the other at three levels, it is preferable to choose the variable with the stronger effect as the first factor. In this case, the five-level factors was chosen for $-\log[\text{NaOH}]$. The experimental data obtained fitted the function

$$A = 544.86 - 277.33Y - 372.38X + 115.15XY + 33.13Y^2 + 76.85X^2$$

where A = absorbance, $X = -\log[\text{NaOH}]$ and $Y = 10^5 \times [\text{Quinizarin}]$ (concentrations in moles per liter). The application of Lagrange's criterion ($\partial^2 A/\partial X^2 = 153.8, \partial^2 A/\partial Y^2 = 66.2, H(X, Y) = -3089.5$) indicated the presence of a saddle point. As no maximum was found, the absorbance maximum variation directions at points 5 and 7 (Fig. 2) were calculated, since this appeared to be the direction of increase in the response. The maximum variation directions of response found for each point were 29.77° and 31.2°. Because the two directions are almost parallel, a new experimental design is carried out in the referred direction (Fig. 2).

The proposed Doehlert design II was centred on the point $X = 3.0$, $Y = 3.0$, and the new experimental results obtained are shown in Table 2 (Design II). The equation which fits the new experimental values is

$$A = -1049.59 - 283.82Y + 11.4076X + 25.76XY + 49.59Y^2 - 198.25X^2$$

The application of Lagrange's criterion indicated the presence of a saddle point ($\partial A^2/\partial X^2 = -396.5$, $\partial A^2/\partial Y^2 = 99.2$, $H(X, Y) = 39\,991.9$). The absorbance maximum variation directions at the (11) and (12) points were calculated (Fig. 2). The maximum variation directions of responses found for each point were 44.47° and −68.91°. The lines drawn from the points, with the calulated directions, intersected at a coordinate point $X = 3.15$ and $Y = 4.47$.

Table 2 (Design III) shows the proposed Doehlert design III, centred on $X = 3.33$ and $Y = 4.00$, and the experimental results obtained. The contour diagram of the response surface is shown in Fig. 2. The new equation fitted to the experimental values is

Table 2
Experimental values used in the simultaneous optimization of Quinizarin and NaOH concentrations (mol l^{-1}), from Doehlert designs I, II and III ($[Li^+] = 1.2 \times 10^{-3}$ M)

Doehlert design	Experiment	$10^5 \times$ [Quinizarin]	−log[NaOH]	Absorbance
(I)	1	1.0	1.67	0.075
	2	1.0	2.33	0.129
	3	2.0	1.33	0.80
	4	2.0	2.00	01.46
	5	2.0	2.67	0.281
	6	3.0	1.67	0.170
	7	3.0	2.33	0.376
(II)	8	2.0	3.33	0.423
	9	3.0	3.00	0.415
	10	3.0	3.67	0.276
	11	4.0	2.67	0.446
	12	4.0	3.33	0.622
(III)	13	4.0	4.00	0.179
	14	5.0	3.00	0.490
	15	5.0	3.67	0.031

$$A = -13\,029.40 + 2669.22Y + 5318.84X - 238.81XY - 239.41Y^2 - 696.71X^2$$

The application of Lagrange's criterion indicated the presence of a maximum ($\partial A^2/\partial X^2 = -1393.4$, $\partial A^2/\partial Y^2 = -478.8$, $H(X, Y) = 610\,172.6$), which is the required optimum and corresponded to 4.0×10^{-5} M Quinizarin and 7.4×10^{-4} M NaOH.

The pK_1 and pK_2 values of Quinizarin in water are 9.35 and 11.78 respectively [38] while in 9:1 dimethylsulfoxide:water they should be slightly higher due to the lower value of the dielectric constant (46.5) of this proton-donor solvent with respect to that of water (78.3) [39]. The optimum working value of apparent pH found (≈ 10.9) shows that only the first acid disociation occurs and the corresponding mono-anion is the reactive species.

3.4. Effect of concentrations of salts

The effect of increasing concentrations (10^{-4}–0.1 M) of different salts (Na_2CO_3, NaCl, KCl) on the abosorbance of the chelate, measured at 601 nm against a reagent blank, was studied. A maximum absorbance was obtained for a 10^{-3} M concentration of Na_2CO_3.

3.5. Effect of temperature

When the solution was thermostated between 10 and 50°C the absorbance of the chelate showed a linear decrease (temperature coefficient = -8.1×10^{-3} absorbance units $°C^{-1}$), while the absorbance of the reagent blank appeared to be constant. A working temperature of 25°C was chosen.

3.6. Other variables

The effect of the order of addition was studied, and found to be insignificant. Measurements were stable 30 min to 2.5 h after the preparation.

2.5. Calibration curve (Beer's law)

Experiments indicated that the Beer's law was obeyed for lithium concentrations up to 250 ppb, while higher concentrations yielded a non-linear response. New factorial designs (2^2) were performed in order to check the optimized NaOH

Table 3
Statistics and performance characteristics of the analytical methods from the calibration data set in aqueous and serum matrices

Parameter	Matrix	
	Water	Serum
Statistics		
Residual standard deviation (a.u.)[a]	3.909	2.883
Intercept (a.u.)	-2.51×10^{-3}	1.55×10^{-3}
Slope (a.u. per ppb Li)	0.63×10^{-3}	0.36×10^{-3}
% Lack-of-fit P value	39.9	62.4
Performance[b]		
Linearity (%)	98.3	98.3
Analytical sensitivity (ppb Li)	6.2	8.0
Detection limit (ppb Li)	13.2	13.3
Determination limit (ppb Li)	44.1	44.3
Precision (relative standard deviation, %):		
50 ppb Li	9.0	7.4
100 ppb Li	4.0	3.9
150 ppb Li	2.6	2.6
200 ppb Li	2.0	2.0
250 ppb Li	1.8	1.8

[a] a.u.: absorbance units.
[b] Calculated as indicated in Ref. [41].

and Quinizarin concentrations given above for a lithium concentration of 250 ppb. The result indicated that both values were suitable for calibration.

The calibration linearity was tested by "lack-of-fit" statistical F-test [40]. Three replicates for the aqueous matrix and five for serum of 0, 50, 100, 150, 200 and 250 ppb lithium standard solution were taken in order to set up the calibrations.

3.8. Analtyical performance characteristics

The main statistical and performance characteristics, calculated from the calibration data set [41], are shown in Table 3. The IUPAC detection limits [42], calculated from 10 replicates of the reagent blank, were 17.6 ppb and 14.5 ppb for aqueous medium and serum respectively.

The ruggedness [43] of the new analytical method for the determination of 100 ppb Li^+, for variations of $\pm 10\%$ in procedure temperature and concentrations of Quinizarin, NaOH and Na_2CO_3 and for a variation of $\pm 5\%$ in the proportion of water, was studied using a 2^{7-4} saturated factorial design with two dummies. The method was rugged for every variable and no interactions between tested variables were found.

3.9. Effect of diverse ions

The effect of various ions on the determination of 50 ppb Li^+ in aqueous medium was studied, setting the tolerance limit at an error of $\pm ts_R$ [44] over the predicted value for the absorbance of the chelate measured against a reagent blank (t = one-tail Student t value for n-2 degrees of freedom and an α value of 0.05; S_R, standard deviation of the analytical response, predicted for the tested analyte concentration, obtained from the calibration data set). The tolerance limits of various ions are shown in Table 4. Note that the tolerance limits of some ions are low. Thus, in the case of a complex matrix, and as blood serum, a new calibration is required.

Table 4
Tolerance concentrations of several ions in the determination of 50 ppb Li^+

Anion[a]	Tolerance (ppb)	Cation[b]	Tolerance (ppb)
Cl^-	>50 000	K^+	500
I^-	10 000	Mg^{2+}	50
SO_4^{2-}	5000	Zn^{2+}	5
Br^-, NO_3^-	1000	Fe^{3+}	2.5
SiO_3^{2-}	500	Cu^{2+}, Ca^{2+}, Pb^{2+}	1
F^-, PO_4^{3-}	100		
$C_2O_4^{2-}$	10		

[a] All the test solutions were prepared from the sodium salts. (The tolerance concentration of Na^+ ion, calculated from the tolerance concentration of the Cl^- ion, is >32 000 ppb.)
[b] All the test solutions were prepared from the corresponding nitrates.

3.10. Application of the proposed method to real samples

3.10.1. Drugs

The basic method, as described, was applied for the determination of lithium in various pharmaceutical preparations, using flame photometry as the reference method. Results in Table 5 show the values found from both methods and the P value of the corresponding Welch t-test [45].

3.10.2. Blood serum

An accurate volume of standard was added to three different samples of serum, so that the final concentration of Li^+ was of the order of the therapeutic levels (approximately 4–8 ppm). Table 6 shows the recovery of the added lithium. The pooled recovery was 96.7%.

Table 6
Recovery of added Li^+ in different serum samples

Serum	Added (ppm)	Found (ppm)	% Recovery
1	4.00	3.24	81.0
	4.00	3.24	81.0
	4.00	2.69	67.3
	4.00	3.93	98.3
	4.00	3.79	94.8
2	5.00	5.32	106.3
	5.00	5.45	109.1
	5.00	5.59	111.8
	5.00	5.18	103.5
	5.00	5.45	109.1
3	7.00	8.21	117.4
	7.00	7.52	107.5
	7.00	6.70	95.7
	7.00	7.52	107.4
	7.00	7.38	105.4

A t-test, which compares the mean value against a reference value, was carried out in order to check if this value was significantly different from 100%. The statistic used, $t = (\bar{\Re} - 100)\sqrt{n}/s_{\Re}$ ($\bar{\Re}$, pooled recovery; $s_{\Re}$, recovery standard deviation), was obtained from the statistic for the comparison of the means of paired samples [46]. The following data were obtained: $\bar{\Re} = 99.4$; $s_{\Re} =$

Table 5
Determination of lithium in various drugs using flame photometry as the reference method and the proposed spectrophotometric method

Drug	Flame photometry		Proposed method		% P value[b]
	$c \pm s_c$[a]	n[b]	$c \pm s_c$[a]	n[a]	
Otogén (mg Li_2CO_3 per tablet)	18.0 ± 0.1	9	18.4 ± 0.3	9	1.1
Plenur (mg Li_2CO_3 per tablet)	335 ± 4	9	340 ± 7	8	8.7
Glucosor-Litio (mg Li gluconate per ml)	3.28 ± 0.02	3	3.2 ± 0.1	3	31.1

[a] Mean and standard deviation of n determinations.
[b] P value for Welch's t-test.

15.783; $t = 1.605$; d.o.f. = 14, P value = 93.1. The high P value shows that the difference between the pooled recovery and 100 is solely due to random error, and therefore the method does not show systematic error.

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