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+Spectrophotometric determination of Ampicillin and Lansoprazole in pure forms and in their pharmaceutical Formulations

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ABSTRACT
Accurate, rapid and economical methods were used to estimate the two drugs: ampicillin and lansoprazole, using the reagent "4-chloro-7-nitrobenzofurazane" (NBD-Cl) in the alkaline medium, these methods depend on the formation of colored products between those drugs and the chromogenic reagent (NBD-Cl). at (pH 10.8) it has the highest absorption at 468 nm for Ampicillin (AMP), as well as a yellow colored product and at (pH 11.3) and has the highest absorption at 470 nm for Lansoprazole (LPZ), Beer's law was applied in a range of concentrations (3.6-32.4), (3.6-24) µg/ml, with an molar absorptivity (6.908×10^3L/mol. cm) (1.086×10^4L/mol. cm), and the correlation factor (0.9961, 0.9984) respectively, and the limits of detection were (1.511µg/ml), (0.588µg/ml), respectively. The proposed methods have been successfully applied to estimate these drugs in their pure form and in their pharmaceutical preparations such as injections and capsules in ampicillin and lansoprazole respectively

Keywords: spectrophotometric, Ampicillin, lansoprazole, NBD-Cl.

1- Introduction

1-1 Ampicillin (AMP) in (Fig1[a]). A beta-lactam antibiotic using frequently to remedy bacterial infections since 1961. It is an important part of the amino penicillin family and is roughly equivalent to amoxicillin in terms of spectrum and activity scale. AMP a robust antibiotic with relatively short-term stability in water solutions [1] and has been used clinically to treat a wide range of bacterial infections [2]. With the injection, ampicillin is spread rapidly and broadly, causing in a condensation of the drug in bile [3] and then excreted into the gut to cause disruption of the normal intestinal micro flora by reducing the main flora and increasing the presence of yeast as well as encourage a high risk of Clostridium colitis [4]. There are several analytical procedures available in the literature for the analysis of ampicillin, such as Fourier-transform Infrared Spectroscopy (FT-IR)[5], (HPLC)[6], (HPLC)-ultraviolet (UV) detector [7], HPLC-fluorescence detector (FLD) [8], reverse-phase (RP)-HPLC-FLD [9], LC-MS/MS [10] and UPLC-MS/MS [11] analyses.

1-2 Lansoprazole (LPZ) is a substituted benzimidazole, chemically known as methyl-4-(2,2,2-rifluroethoxy)-2pyridyl]methyl]sulfinyl]benzimidazole (Fig1[b]). It is a proton pump inhibitor[12]. LPZ inhibits the final step in gastric acid secretion, and stimulus-independent acid secretion is suppressed, and the last inhibited both basal and stimulus acid. It has exceedingly used in the treatment of benign gastric ulcer associated with H. pylori, duodenal ulcer, and reflux esophagitis, being It has a significant inhibitory effect than omeprazole. LPZ is a toxicant acid and is therefore formulated as capsules containing mainly enteric-coated pellets to prevent gastric decomposition and improve systemic bioavailability. [13].

There are several analytical methods to determine the Lansoprazole in pharmaceuticals and biological fluids, for this purpose was achieved through spectrophotometry [14], Potentiometry [15], Liquid chromatography/tandem mass spectrometry (LC–MS/MS) [16], Electrophoresis [17], Spectrofluorimetry [18], Polarography [19], Voltammetry [20], and HPLC [21] have been used.

1-3 (NBD-Cl), (Fig.1[c]) a fluorogenic reagent for the detection of amines [22] and for spectrophotometric calculation of compounds [23,24]. Thio compounds form highly colored products in an alkaline medium with NBD-Cl which is used for their colorimetric determination [25]. **NBD-Cl** is required to make analytical methods using low-cost techniques. Ultraviolet - Visible spectrophotometry is still considered a suitable and economical technique for regular analysis of drugs in pharmaceutical formulations. Therefore of the Mentioned above reasons, it has become to develop a quantitative method for the determination of the investigated Ampicillin and Lansoprazole with NBD-

Cl, which may be used for their analysis in pure forms and pharmaceutical formulations.

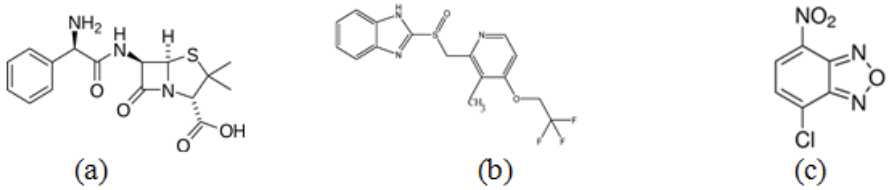


Fig. (1): Chemical Structures of (a) Ampicillin (b) Lansoprazole (c) NBD-Cl reagent

2- Aim of the study

The goal of this study is to find "simple, fast and economical" spectrophotometric techniques for the determination of Ampicillin and Lansoprazole, with "chromogenic reagent NBD-Cl" in an alkaline environment, and the forming compound was researched by job method and the mole-ratio method, and to find if those methods are suitable for the study of the two drugs in pharmaceutical preparations (Injections and capsules).

3- Experimental

3-1 Apparatus

(Single beam from Genesys UV 10) Ultraviolet-Visible spectrophotometer, (InoLab pH/INO735) pH meter from Jenway 3310, (Kern 770GS/GJ) Balance from Sartorius BL210S, Oven manufactured by Memmert, Schutzart .

3-2 Materials

Ampicillin %99, Lansoprazol %99 from (Samarra Drug Industry Samarra-Iraq), "4-Chloro-7-nitrobenzofurazan (NBD-Cl)" %98 (Solarbio), (NaOH) %98 from (GCC), Ethanol %99.9.

3-3 Solutions

3-3-1 Ampicillin Stock solution (1000 µg/ml): precisely (0.1000 gm) of (AMP) "standard" has been dissolved in (100 ml) water.

3-3-2 Lansoprazol Stock solution (1000 µg/ml): precisely (0.1000 gm) of (LPZ) "standard" has been dissolved in (100 ml) ethanol.

3-3-3 NBD-Cl (5×10^{-3}M): has been made with (0.1 gm) of NBD-Cl in (100 ml) ethanol.

3-3-4 NaOH (1M): has been made with (4 gm) of NaOH in (100 ml) distilled water

3-3-5 Magnesium stearate;Cellulose; Mannitol; Magnesium carbonate.

Solutions: a concentration of (1000 µg/ml) has been made by dissolving (0.1000) gm in 100 ml of the appropriate solvent.

4- Procedures

4-1 Ampicillin: A 2 ml from ($5x10^{-3}$ M) of (NBD-CL) was carried into 25ml "volumetric flask and 0.5 ml of (1M NaOH) followed by adding 0.5ml from (300 µg/ml) of (AMP). After the passing of 15 minutes the rest of the volume was supplemented to volume by distilled water and was measured at 468 nm with reagent Blank.

4-2 Lansoprazol: A 3 ml from ($5x10^{-3}$ M) of (NBD-CL) was carried into 25ml "volumetric flask and 0.5 ml of (1M NaOH) followed by adding 0.5ml from (300 µg/ml) of (LPZ). After the passing of 15 minutes the rest of the volume was supplemented to volume by distilled water and was measured at 470 nm with reagent Blank.

4-3 stoichiometric ratio procedures

The equivalence for the reaction of these drugs and the Blank have been calculated by carrying out "molar ratio" and "continuous variation methods". In these methods, "equimolar" solutions of (AMP), (LPZ) and "NBD-Cl" (2×10^{-3}M and 1×10^{-3}M), were used. Varying aliquots of "NBD-Cl" were added to constant volumes of drugs solutions (0.5 ml from AMP and 0.5 ml from LPZ), the absorbance and the final volumes (25ml) were measured at 468 and 470 nm for two color products opposite the "reagent blank treated similarly". While in the latter method, a series of AMP-NBD-Cl and LPZ-NBD-Cl solutions were kept at (5ml) (0:5, 0.5:4.5, 1:4, 1.5:3.5, 2:3, …… 5:0).

4-4 Applications of Mole-ratio and Job methods:

A- We used 0.1gm of (AMP) Injection, weighed, and averages weights were computed and dissolved in 100 ml of distilled water to obtain the final concentration of 1000 µg/ml

B- We used "ten capsules" for (LPZ), weighed, and averages weights were computed. These capsules were grinded into exact powder. A precisely weighed 0.1 gm of powder were transferred into a beaker and they were shaken with 50 ml of ethanol and filtered. The filtrates and the washings were collected in a 100ml "volumetric flask". This filtrate and the washing were diluted up to the mark with solvent to obtain final concentration as 1000 µg/ml.

The suggested methods were successfully implemented for the determination of (AMP), and (LPZ) in various commercial Injection and Capsules.

5- Results and Discussion

Absorption spectra of "AMP-NBD-Cl",and"LPZ-NBD-Cl"systems with blank reagent in an alkaline medium at nominal temperature (25^{o}C) producing an "yellow colored products" for each drugs where absorbs maximally at 468 nm for (AMP),470nm for(LPZ),(Fig.2,3) and reagent blank against ethanol (Fig. 4).

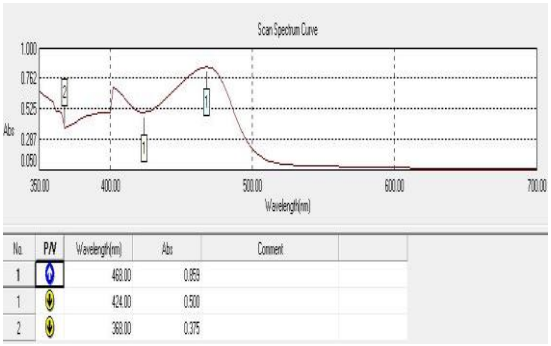


Fig. (2): Absorption of AMP-NBD-Cl system against blank

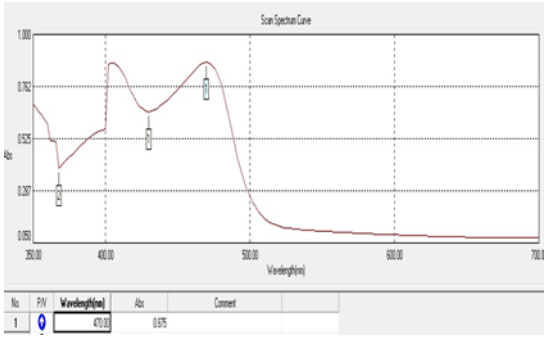


Fig. (3): Absorption of LPZ-NBD-Cl system against blank

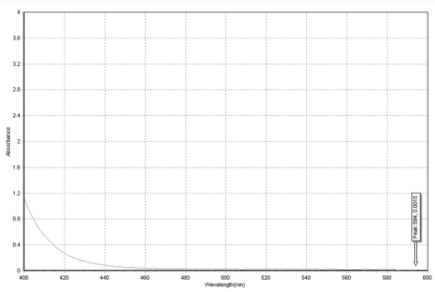


Fig. (4): Absorption of reagent blank against ethanol

5-1 Optimum conditions

To establish optimum conditions, it required to creation of "colored product with maximum stability and sensitivity", the influence of volumes of "NBD-Cl", addition of "alkaline intermediate", "reaction time" and the "stability of colored products" were set up at "room temperature (25^0C) ".

5-1-1 Effect of reagent volumes

The effect of reagent concentration on the reactions w as examined at "room temperature". The reactions of (AMP), (LPZ) with reagent were to rely on the concentrations of "NBD-Cl". So, its concentrations were studied by different volumes from (0.2 to 3.0 ml) with (AMP) and (1.0 to 4.5 ml) with (LPZ) of (0.005M) NBD-Cl, while the (AMP), and (LPZ) concentrations were maintained constant at 6 µg/ml for both drugs (As a final concentration from 300 µg/ml as a primary concentration) for each.

The colour intensity was found to increase with the addition of NBD-Cl up to a particular concentration and then either decrease or remain steady, When the amounts of absorption were achieved, the maximum absorption rate was NBD-Cl were (2.0 and 3.0) ml, of 0.005 M, Therefore, these concentrations were used to prepare calibration curves.

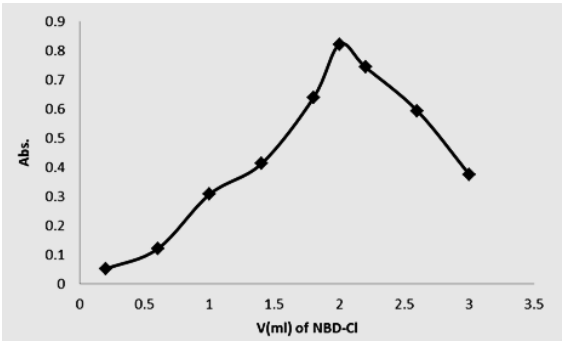


Fig.(5): Effect of vol. of NBD-Cl on AMP-NBD-Cl product

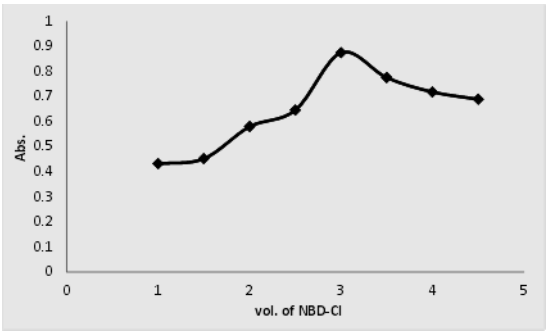


Fig.(6): Effect of vol. of NBD-Cl on LPZ- NBD-Cl product

5-1-2 Effect of pH

An alkaline medium was required, because these drugs does not reacts with "NBD-Cl" In acidic medium, the findings showed that absorption at pH < 8 was close to 0, these drugs had trouble reacting with 'NBD-Cl.' in acidic intermediate. Different concentrations from NaOH were tested. Best results were at higher concentrations of NaOH (1M), with pH 10.8, 11.3 for (AMP), and (LPZ) colour products, respectively, As illustrated in Fig. (7, 8).

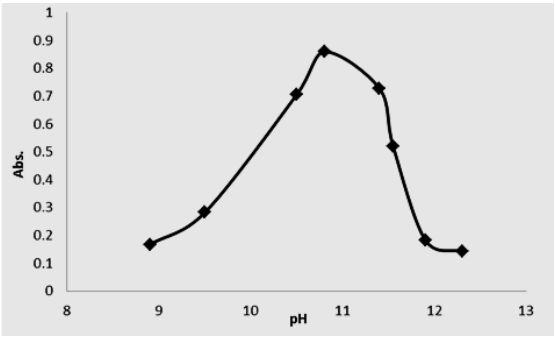


Fig.(7): Effect of pH on AMP-NBD-Cl product

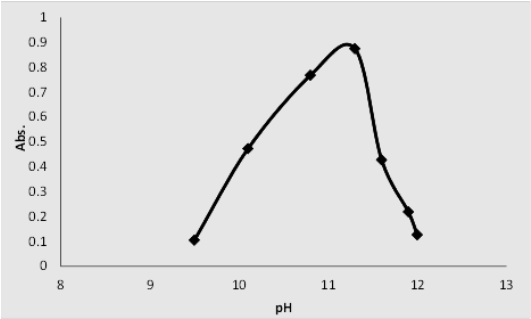


Fig.(8): Effect of pH on LPZ-NBD-Cl product

5-1-3 Time Effect:
Under the "optimal conditions", the effect of time reaction of (AMP), and (LPZ) with reagent in "alkaline medium" were studied, and the products stable remained to 50 min. for (AMP), and to 45 min for (LPZ), As illustrated in Table (1, 2).

Table (1): Effect of Time on products constant (AMP)

Time (Min)	Abs. of (AMP)
0	0.859
5	0.856
10	0.858
15	0.860
20	0.862
25	0.857
30	0.863
35	0.863
40	0.856
45	0.860
50	0.862
55	0.833
60	0.826

Table (2): Time effect on products constant (LPZ)

(Min) Time	Abs. of (LPZ)
0	0.875
5	0.871
10	0.873
15	0.880
20	0.878
25	0.878
30	0.876
35	0.873
40	0.872
45	0.874
50	0.862
60	0.855

5-1-4 Additives effect: Effects of additives on the composition between products (LPZ) with NBD-Cl The reagent was examined and no effect was observed, as in the Table (3). There are no additives in the pharmaceutical preparation for (AMP) which was in the form of injection.

Table (3): Effect of additives on (LPZ) product

Additives	Add. (µg/ml)	%RE	Add. (µg/ml)	%RE
Magnesium Carbonate	40	0.914	60	1.829
Cellulose	40	-0.457	60	-2.171
Mannitol	40	3.086	60	4.229
Magnesium Stearate	40	-0.229	60	-3.771

5-1-5 The reactions equivalent: Under the "optimum conditions", (temperature, cons. of NBD-Cl, pH, time) "the stoichiometry" of the reactions between (AMP), and (LPZ) with Reagents were analyzed by methods of mole-ratio and continuous variation. The equivalence between reagent and these drugs were 2:1 for (AMP), and 1:1 for (LPZ) (Figs. 9, 10, 11, 12)

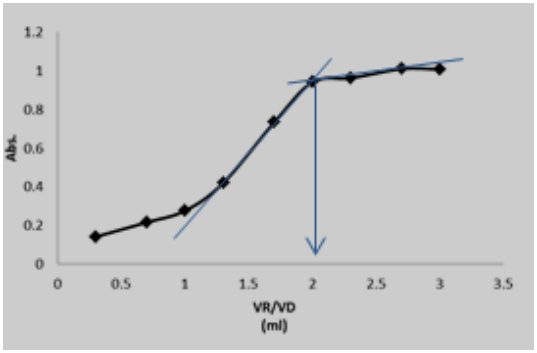


Fig.(9):Mole-ratio method of AMP

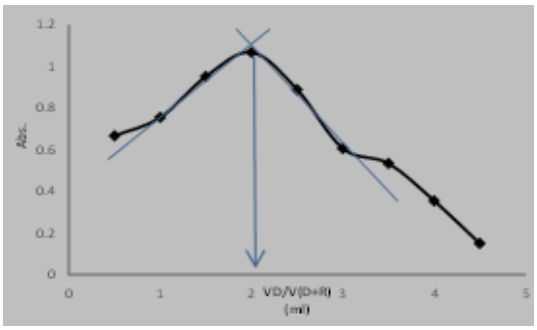


Fig.(10):Continuous variation method of AMP

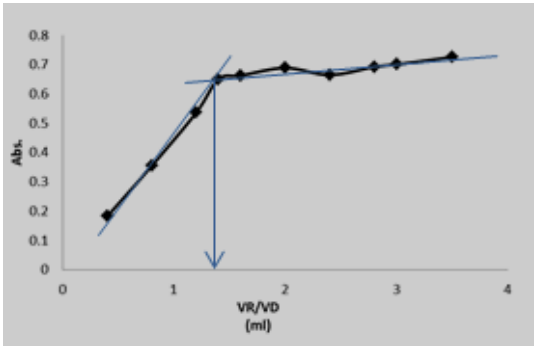


Fig.(11):Mole-ratio method of LPZ

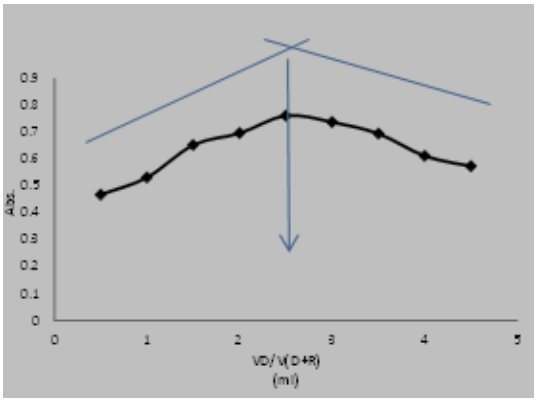


Fig. (12):Continuous variation method of LPZ

5-1-6 Calibration curves: The calibration curves for (AMP), and (LPZ) standard forms through correlation with NBD-Cl showed the linearity at concentrations

ranges of (3.6-32.4 µg/ml), and (3.6-24 µg/ml), respectively, as shown in Figs. (13, 14).

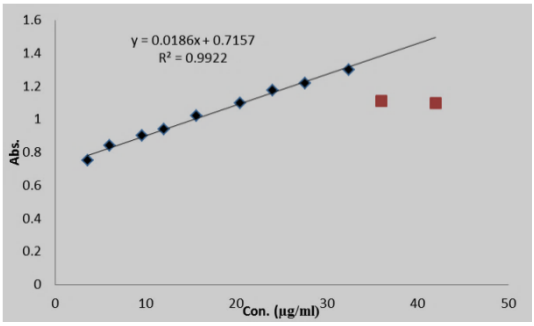


Fig. (13):Calibration curve of AMP product

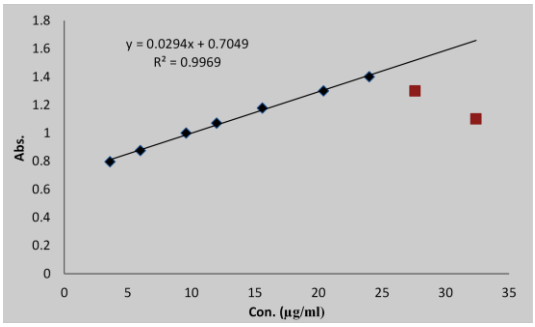


Fig. (14): Calibration curve of LPZ product

5-1-7 Construction of curves for calibration:
In Table (4), calibration curves were built according to the optimum conditions.

Table (4) Optical characteristics of spectrophotometric determination (AMP) calibration curves, and (LZP) by NBD-Cl reagent

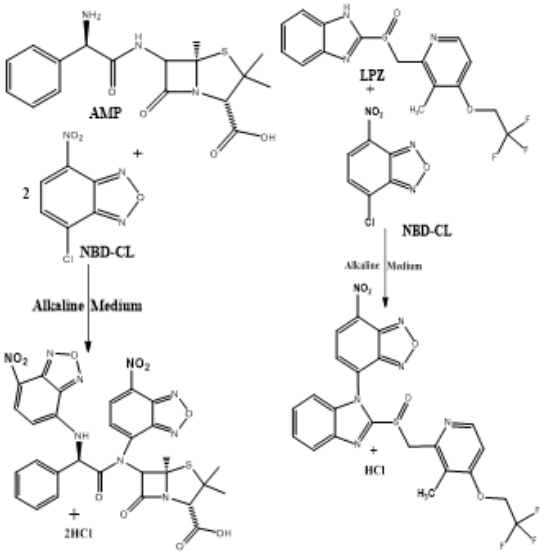
Parameter	(AMP)	(LZP)
λmax.(nm)	468	470
Beer's law (µg/ml)	3.6-32.4	3.6-24
Molar absorptivity(L/mol.cm)	6.908×10^{3}	1.086×10^{4}
Correlation coefficient (r)	0.9961	0.9984
Limit of Detection (µg/ml)	1.511	0.588
Slope	0.0186	0.0294
Intercept	0.7157	0.7049
%RSD	1.132	0.624

5-1-8 Application of the methods proposed
The results of the determination of (AMP) and (LZP) in pharmaceutical preparations are shown in Table (5). (Injections and Capsules).

Table (5): Determination of (AMP), and (LPZ) by spectrophotometric process in commercial injections and capsules

Drug	Content(µg/ml) declared	Found(µg/ml) by proposed method	%RE	%Recovery
(AMP) 500 mg				
TROGE	9.6	9.4	-2.08	97.92
Stricillin	15.6	14.9	-4.48	95.51
(LZP) 30 mg				
Degastrol	9.6	9.3	-3.13	96.88
LpZ-30	15.6	15.1	-3.21	96.79

5-1-9 Suggested reactions: Suggested ractions can be as in the following equations: (the drugs are associated with the reagent through the amine groups) [26,27]:



6- Conclusions

These methods mentioned in this study are 'simple , easy, convenient' and, unlike many other documented methods, do not require special working conditions. The procedures showed a shorter time for reactions, stable colored species with cheap reagents. The analysis can be made at room temperature and does not require steps of heating. For the determination of (AMP) and (LPZ) in pharmaceutical preparations (Injections and Capsules), the methods suggested may be applied.

7- References

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التقدير الطيفي للامبسيلين واللانسوبرازول بأشكالها النقية وفي مستحضراتها الصيدلانية

رويده فرمان صالح ، قبس ناجي رشيد

قسم الكيمياء ، كلية التربية للعلوم الصرفة ، جامعة تكريت ، تكريت ، العراق

الملخص

تم استخدام طريقة حساسة وسريعة واقتصادية لتقدير العقارين: الأمبيسلين واللانسوبرازول، باستخدام كاشف "4–كلورو–7–نيتروبنزوفورازان" (NBD-Cl) في الوسط القلوي، وتعتمد هذه الطرائق على تكوين نواتج ملونة بين تلك الأدوية والكاشف الكروموجينيي (NBD-Cl). وظهر ناتج اصفر اللون عند (pH 10.8) وكان أعلى امتصاص عند 468 نانومتر للأمبيسيلين، وعند (pH 11.3) كان أعلى امتصاص عند 470 نانومتر للانسوبرازول، انطبق قانون بير في مدى من التراكيز (3.6–32.4)، (3.6–24) مكغم/مل، وكانت الامتصاصية المولارية (6.908×10^{3} لتر/مول.سم)، (1.086×10^{4} لتر/مول.سم)، وعامل الارتباط (0.9961، 0.9984) على التوالي، وحدود الكشف كانت (1.511 مكغم/مل)، (0.588 مكغم/مل)، على التوالي. تم تطبيق الطرائق المقترحة بنجاح لتقدير هذه الأدوية في شكلها النقي وفي مستحضراتها الصيدلانية مثل الحقن والكبسولات في الأمبيسلين واللانسوبرازول على التوالي.