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Spectrophotometric determination of lisinopril in tablets using 1-fluoro-2,4-dinitrobenzene reagent

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Abstract

A spectrophotometric method for the determination of lisinopril (LN) in single and multicomponent tablets also containing hydrochlorothiazide (HCT), based on the derivatization reaction with 1-fluoro-2,4-dinitrobenzene (FDNB, Sanger reagent) is described. Aqueous solutions of LN ($4.5–27.2 \times 10^{-5}$ M) react with FDNB (in acetonitrile) at pH 8.2 (borate buffer) in dark at 60 °C for 45 min. After acidification with HCl to decolourize 2,4–dinitrophenolate (the alkaline hydrolysis product of FDNB), the LN-derivative is measured at 356.5 or 405.5 nm (only at 405.5 nm if HCT is present). The calibration curves are linear ($r > 0.996$ at both wavelengths) with a between days precision of slopes of 1.8 and 2.3% at 405.5 and 356.5 nm, respectively. The quantification limit is 3.49×10^{-5} M (0.014 mg) at 405.5 nm and 5.69×10^{-5} M (0.023 mg) at 356.5 nm. The accuracy and precision of the method were evaluated with the analysis of synthetic mixtures (Er%: 0.30–0.60 and 0.27–1.00 at 405.5 and 356.5 nm, respectively; RSD%: 0.48–0.92 and 0.35–0.51 at 405.5 and 356.5 nm, correspondingly; recovery%: 99.2–100.4 at 405.5 nm and 97.9–104.3 at 356.5 nm). Results obtained from the analysis of commercial preparations with the proposed method are in good agreement with those obtained with the official HPLC method (% relative difference 0.2–2.5%). The developed method can be used for rapid routine analysis for content uniformity, dissolution profile studies and assay of pharmaceutical preparations.

Keywords: Spectrophotometry; 1-Fluoro-2,4-dinitrobenzene; Lisinopril; Hydrochlorothiazide; Derivatization; Tablets

1. Introduction

Lisinopril (LN), (2*S*)-1-[(2*S*)-6-amino-2[[(1*S*)-1-carboxy-3-phenylpropyl]amino] hexanoyl]pyrrole-2-carboxylic acid, is a synthetic dipeptide (lysyl–proline) used for the treatment of hypertension, for congestive heart failure and after myocardial infraction [1–3]. It reduces both angiotensin and aldosterone plasma concentrations through the inhibition of angiotensin converting enzyme [1–3]. Several methods have been presented in the literature for the determination of LN in pharmaceutical preparations [4–18]. These include HPLC [5,9,14,17,18], GC [10,11], spectrophotometry [5,12,15], derivative spectrophoto-

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metry [4,8,9,13,16], spectrofluorimetry [5,12], capillary electrophoresis [6,7] and HPTLC-densitometry [4]. The official method, described in national pharmacopoeias, for the assay of LN in tablets and raw material is based on HPLC with UV detection [19] and potentiometric titration [20] with 0.1 M NaOH, respectively. The combination of LN and hydrochlorothiazide (HCT) as a multicomponent tablet has been recently introduced in the market for the treatment of hypertension [3] and their assay has been achieved by HPLC with UV detection [17], derivative UV spectrophotometry [4,16] and HPTLC-densitometry [4].

LN shows very low absorptivity in UV spectrum (maxima at ~ 246, ~ 254, ~ 258, ~ 261 and ~ 267 nm with respective $A_{1\%}^{1cm}$ values of ~ 4.0, ~ 4.5, ~ 5.1, ~ 5.1 and ~ 3.7 [18]). Therefore several derivatization reactions have been used to develop spectrophotometric methods in the UV–Vis region. Chloranil, dichlone, Hantzsch reaction [12] and hypochlorite/phenylhydrazine [5] have been used to develop such methods for the analysis of pharmaceutical preparations. 1-Fluoro-2,4-dinitrobenzene (FDNB) (Sanger's reagent) is a very useful reagent to introduce a chromophore group in amines, phenols and thiols by nucleophilic substitution reactions [21–23]. This reagent has never been tried for LN determination and the aim of this investigation was: (a) to study the possibility of derivatizing the LN molecule with FDNB and optimize the reaction conditions, (b) to study the effect of common excipients on the derivatization reaction and (c) to develop a spectrophotometric method for the assay of LN in single or multicomponent formulations with HCT.

2. Materials and methods

2.1. Apparatus

A Hitachi 2200 UV–Vis spectrophotometer, with 1-cm quartz cell, was used to obtain spectra and absorbance measurements. A Metrohm Herisau pH–meter model E350B was used for buffer preparation.

2.2. Materials and reagents

All materials and reagents used were of analytical grade. LN dihydrate (purity 99.9%) was kindly provided by GenePharma (Greece) and hydrochlorthiazide (purity 100.4%) was kindly provided by Gap (Greece). Zestril® (produced by Zeneca, Greece) and Z-Bec® (produced by Gap, Greece) tablets containing 5, 10, 20 mg LN as anhydrous base were purchased from local market. Zestoretic® (produced by Cana, Greece), and Prinzide® (produced by, Vianex, Greece) tablets containing 20 mg LN as anhydrous base and 12.5 mg HCT, were also purchased from local market. FDNB (purity 100%) was purchased from Fluka. Common excipients (mannitol, starch maize, magnesium stearate, dibasic potassium phosphate) were obtained from various companies. Acetonitrile was HPLC grade (Lichrosolv®) and was obtained from Merck.

2.3. Solutions

All solutions were prepared in distilled, deionized water. Standard stock solution of LN 5.44×10^{-3} M (2.21 g/l) expressed as anhydrous base was prepared by dissolving 120.1 mg of LN dihydrate (corresponding to 110.3 mg of LN anhydrous base) in 50.00 ml of water. Stored in a refrigerator it was stable for 6 months. Working standard solutions in the range of 4.53×10^{-5} M (0.02 g/l)–$2.72 \cdot 10^{-4}$ M (0.11 g/l), were prepared by appropriate dilution of standard stock solution with water.

Stock solution of HCT 1.68×10^{-4} M (0.05 g/l) was prepared in water.

Stock solution of FDNB 0.0195 M (3.63 g/l) was prepared by dissolving 181.7 mg of the reagent in 50.00 ml of acetonitrile. This reagent should be handled carefully since it is a skin irritant. Stored in a refrigerator it was stable for 4 months. Working solutions in the range of 0.0030 M (0.56 g/l)–0.0150 M (2.79 g/l) were prepared by appropriate dilution of the standard stock solution with acetonitrile.

A 1.0 M hydrochloric acid solution was prepared in water by dilution of a 12 M hydrochloric acid commercial solution.

A borate buffer 0.050 M containing KCl 0.050 M solution (pH 8.2) was prepared by dissolving the appropriate amount of H_3BO_3 and KCl in water and adjusting the pH by adding 2.0 M NaOH solution, while checking the pH with the pH meter.

2.4. Sample preparations

2.4.1. Pharmaceutical forms (single or multicomponent tablets)

An accurately weighted mass of powdered tablets (20 tablets) in the range of their mean weight was transferred into a 20-ml beaker, 20 ml of water were added, the suspension was sonicated for 20 min, stirred mechanically for another 10 min, filtered through a paper filter (Whatman No. 42) into a 250-ml volumetric flask and the filtrate was completed to volume with water. From the resulting solution 1.00 ml was used for the derivatization–measurement procedure described in Section 2.5.

2.4.2. Synthetic mixtures

For the validation of the method, synthetic mixtures containing different amounts of LN equivalent to 10.4, 20.3 and 28.9 mg of LN anhydrous base as well as the excipients in amounts encountered in the commercial tablets, i.e. dibasic potassium phosphate (124.1 mg), magnesium stearate (2 mg), mannitol (41.2 mg) and starch maize (31.5 mg) were prepared (in six independent experiments) and treated as described in Section 2.4.1.

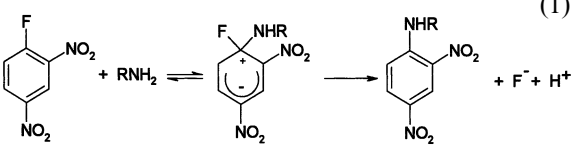
2.5. Derivatization and absorbance measurement

1.00 ml of each of the LN standard or sample solutions was transferred into 10-ml cylindrical glass test tubes with plastic screw cap. To each tube, 400 μl of FDNB 0.0060 M (2400 nmoles) solution in acetonitrile, 1.00 ml borate buffer pH 8.2 and 4.00 ml of acetonitrile were added. After 2-s vortexing the tubes were allowed to stand in dark at 60 °C for 45 min in a covered water bath. After cooling, under tap water, the solution in each tube was acidified with 150 μl of 1.0 M HCl solution, transferred quantitatively to a 10-ml volumetric flask and diluted to volume with acetonitrile (final dilution 1:10). The absorbance of the coloured product was measured at λ_{max} 356.5 and 405.5 nm against a reagent blank (1.00 ml of water was used instead of LN solution). Regression equations of the calibration curves were obtained by least-squares linear regression of the absorbance versus molar concentration data, at 356.5 and at 405.5 nm.

3. Results and discussion

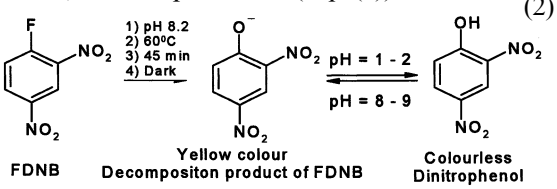
The reaction of FDNB with drugs that own a free primary amine group results in the formation of coloured products [21–23]. This reaction was first introduced by Sanger as a means for determination of the DNA sequence. Recently FDNB has been used for the spectrophotometric determination of norfloxacin [25] in pharmaceutical preparations and for the high performance liquid chromatographic determination of lysine in infant milk [26]. The reaction is a typical nucleophilic substitution subjected to basic and micellar catalysis [21] and proceeds through an intermediate product (Eq. (1)):

(1)



The unreacted FDNB undergoes basic hydrolysis to 2,4-dinitrophenolate (Eq. (2)).

(2)



The reaction was performed in a slightly alkaline environment [24] in order to favour the reaction of LN with FDNB without rapid hydrolysis of the reagent to 2,4-dinitrophenol. FDNB was used in excess to improve the reaction yield. From this reaction a pale yellow coloured LN derivative (LN–FDNB) is formed, along with the alkaline

hydrolysis product of FDNB (dinitrophenolate), which exhibits an intense yellow colour. The spectra of the two products are overlapped rendering impossible the measurement of the analyte derivative. However, by acidification, after the completion of the reaction, the yellow 2,4-dinitrophenolate turns to the uncoloured 2,4-dinitrophenol, allowing the measurement of the LN–FDNB derivative which remains stable and exhibits absorption maxima at 356.5 and at 405.5 nm. A typical spectrum of the LN–FDNB derivative, produced under the experimental conditions described in Section 2.5, is shown in Fig. 1.

The experimental conditions for the derivatization reaction were optimized by the univariate method (changing one parameter at each step). The effect of the experimental parameters studied (time, temperature, FDNB concentration, pH) on the measured absorbance was validated. The optimum time for the completion of the reaction between LN and FDNB and the optimum temperature were 45 min at 60 °C, respectively, and any further increase of these parameters resulted in a plateau of the observed absorbance (Figs. 2 and 3). The concentration of FDNB, studied in the range of 0.0030–0.0150 M, showed no influence on the measured absorbance. A 0.0060 M FDNB solution was finally chosen, allowing a LN/DNFB moles ratio of 1/9–1/53 in the reaction mixture.

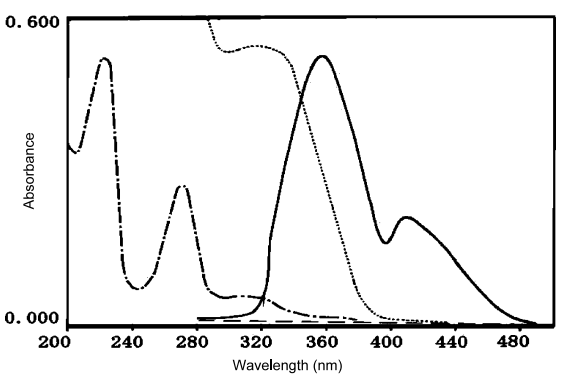


Fig. 1. Absorption spectra derived under the experimental conditions described in Section 2.5. LN–FDNB (—) at final concentration of 1.97×10^{-5} M against the derivatization reaction's blank solution (Derivatization reaction: $C_{LN} = 1.97 \times 10^{-4}$ M, $C_{FDNB} = 0.0060$ M), FDNB reagent (– –) at final concentration of 2.4×10^{-4} M in the derivatization reaction's medium against the derivatization reaction's medium (initial $C_{FDNB} = 0.0060$ M), HCT at final concentration of 1.67×10^{-6} M (– · –) and 1.67×10^{-5} M (· · · ·) against the derivatization reaction's blank solution.

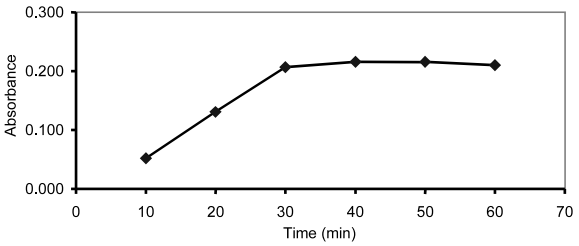


Fig. 2. Effect of time to the yield of the reaction of LN with FDNB at 405.5 nm ($C_{LN} = 1.20 \times 10^{-4}$ M, $C_{FDNB} = 0.0060$ M, pH 8.2, 60 °C, $C_{HCl} = 1.0$ M).

In order to establish the alkaline environment for the reaction, four different means were used, namely a Tris buffer 0.17 M (pH 8), a borate buffer 0.05 M–KCl 0.05 M (pH 8.2), a borate buffer 0.08 M (pH 9.0) and NaOH 0.010 M (pH 12). When Tris buffer was used the decolouration of dinitrophenolate by acidification with 1.0 M HCl was impossible. When 0.010 M NaOH solution was used the rapid hydrolysis of FDNB to dinitrophenolate inhibited the reaction between FDNB and LN. The derivatization reaction was successfully carried out with borate buffer. Examining borate buffer 0.05 M in the pH range 8–10 at both wavelengths, the pH 8.2 was found as optimum (Fig. 4). Using borate buffer pH 8.2 the decolouration step was easily achieved using 1.0 M HCl solution. Any further increase of HCl concentration did not alter the measured absorbances of LN-FDNB. Under the selected optimal conditions, absorbance was linearly related to

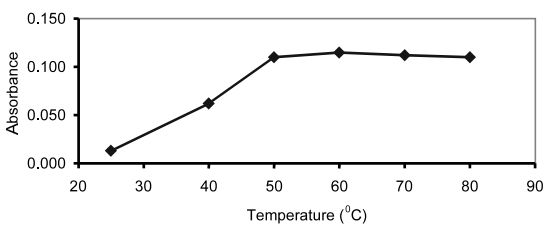


Fig. 3. Effect of temperature to the yield of the reaction of LN with FDNB at 405.5 nm ($C_{LN} = 1.20 \times 10^{-4}$ M, $C_{FDNB} = 0.0060$ M, $t = 45$ min, pH 8.2, $C_{HCl} = 1.0$ M).

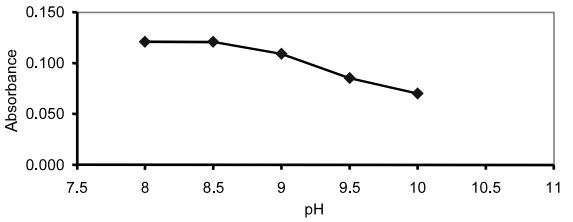


Fig. 4. Effect of pH of borate buffer to the yield of the reaction of LN with FDNB at 405.5 nm ($C_{LN} = 1.20 \times 10^{-4}$ M, $C_{FDNB} = 0.0060$ M, $t = 45$ min, 60 °C, $C_{HCl} = 1.0$ M).

the concentrations of the LN in the concentration range (after dilution) of 4.53×10^{-6} M–2.72×10^{-5} M, resulting in an observed molar absorptivity of 1.40×10^4 and 5.7×10^3 M^{-1} cm^{-1} at 356.5 and at 405.5 nm, respectively.

Data concerning the between days precision of regression equations of the calibration curves by the proposed method is presented in Table 1. The linearity was good ($r > 0.996$) and the line passes through zero (as proven by the Students' t-test). Between days precision of the slopes of the calibration curves was 1.8 and 2.3% at 405.5 and at 356.5 nm, respectively. The observed molar absorptivities of the LN-FDNB are equal to the slopes of calibration curves in which concentration C is expressed after dilution. The quantification limits of the method were estimated from the mean equations of the calibration curves based on the standard deviation of the intercepts. The quantification limit (based on 10 times the SD) was found equal to 3.49×10^{-5} M (0.014 mg) at 405.5 nm and 5.69×10^{-5} M (0.023 mg) at 356.5 nm (initial concentrations and amounts).

The precision and accuracy of the proposed method were evaluated by analyzing three synthetic mixtures containing different amounts of LN and the results obtained are presented in Table 2. The precision of the method was evaluated by estimating the %RSD of the mean absorbances at 356.5 and at 405.5 nm, found from six independent experiments from the synthetic mixtures. As shown (Table 2), in all cases %RSD was less than 0.92%. For the evaluation of the accuracy the amount of LN found using the calibration curve was compared with the amount of LN added in the synthetic mixture and the accuracy was expressed as mean % relative error. As shown (Table 2), in all cases Er% was less than 1%. The accuracy was also tested by recovery calculation by comparison of the absorbances measured from standard LN solutions in the presence and absence of excipients. Recovery ranged from 97.9 to 104.3% and was estimated using one point calibration method. From the results presented in Table 2 it can be concluded that both wavelengths examined are suitable for the quantitative determination of LN in single component LN tablets following the proposed method.

The single component commercial tablets of LN were assayed, using the proposed and the official [19] methods. The assay results, which are presented in Table 3, show good agreement be-

Table 1
Equations of linear regression of calibration curves for the determination of LN by the proposed method

Day	LN $\times 10^{-6}$ M (after dilution)	405.5 nm			356.5 nm		
		Intercept ± SD	Slope ± SD	r^a	Intercept ± SD	Slope ± SD	r^a
1	4.53–27.2	0.006 (±0.002)	5725 (±103)	0.9992	0.001 (±0.002)	13 903 (±475)	0.998
2		0.003 (±0.004)	5641 (±211)	0.997	0.008 (±0.004)	13 948 (±191)	0.9990
3		0.005 (±0.002)	5686 (±134)	0.9997	0.019 (±0.003)	14 530 (±174)	0.9998
4		0.003 (±0.001)	5706 (±57)	0.9997	0.001 (±0.008)	14 219 (±446)	0.9990
5		0.000 (±0.003)	5912 (±190)	0.998	0.000 (±0.010)	13 675 (±550)	0.996
Mean calibration curve		$A = 5734(\pm 104)C + 0.003(\pm 0.002)$			$A = 14055(\pm 328)C - 0.005(\pm 0.008)$		
Mean molar absorptivity (M^{-1} cm^{-1})		5734(±104)			14 055(±328)		

[a] Correlation coefficient.

Table 2
Assay results for the determination of LN in synthetic mixtures

mg of LN anhydrous base	Precision ($n = 6$)			Accuracy ($n = 6$)		% Recovery ± SD ($n = 6$)	
	Wavelength (nm)	$\bar{A} \pm$ SD	%RSD	Wavelength (nm)	Er%[a]	Wavelength (nm)	
10.4	405.5	0.0650 ± 0.0006	0.92	405.5	0.60	405.5	99.2 ± 0.8
	356.5	0.1425 ± 0.0005	0.35	356.5	0.46	356.5	104.3 ± 0.4
20.3	405.5	0.1160 ± 0.0006	0.52	405.5	0.30	405.5	100.4 ± 0.4
	356.5	0.2740 ± 0.0014	0.51	356.5	1.00	356.5	97.9 ± 0.5
28.9	405.5	0.1673 ± 0.0008	0.48	405.5	0.38	405.5	100.2 ± 0.4
	356.5	0.395 ± 0.002	0.51	356.5	0.27	356.5	99.0 ± 0.5

[a] Mean absolute value of six independent experiments.

tween the proposed and the official method with relative differences of 0.2–2.5% and statistical equivalence using t-test. The precision of the assays is also very good.

In order to use the proposed method in the assay of multicomponent pharmaceutical preparations containing LN and HCT, the reaction of HCT with FDNB was examined. The experiments showed that HCT could not be derivatized with FDNB and moreover, HCT and LN–FDNB derivative show overlapping spectra in the UV–region of 320–360 nm, at concentrations similar to those existing in commercial multicomponent formulations (derivatization reaction: initial $C_{HCT} = 1.67 \times 10^{-4}$ M, final $C_{HCT} = 1.67 \times 10^{-5}$ M) as shown in Fig. 1. Thus, the simultaneous determination of LN and HCT is impossible with the developed method.

In order to examine whether the determination of LN in multicomponent pharmaceutical formulations is possible in the presence of HCT, the effect of constant amount of HCT on the calibration curve of LN was examined at both wavelengths. The calibration curve at 356.5 nm showed a significant decrease in slope and an increase of intercept than the one obtained in the absence of HCT (Students' t-test has been performed for this comparison) and thus the determination of LN in the presence of HCT was impossible at 356.5 nm. However, the calibration curve's slope and intercept at 405.5 nm in the presence and in the absence of HCT were exactly the same. Therefore, we can support that the method developed is suitable for the determination of LN in multicomponent formulations containing HCT when the absorbance is measured only at 405.5 nm.

Prinzide and Zestoretic tablets containing labelled amounts of 20 mg LN and 12.5 mg HCT, were analyzed by the proposed method ($n = 5$) and LN content was found to be equal to 19.96 ± 0.24 and 20.2 ± 0.16 mg, respectively (99.8 ± 1.2 and 101.0 ± 0.8% correspondingly of the claimed amount).

The previously reported spectrophotometric methods based on derivatization reactions of LN with hypochlorite/phenylhydrazine [5] and chloranil [12] measure the absorbance of the corresponding derivative at 362 and 346 nm, respectively, and therefore are inappropriate for the determination of LN in the presence of HCT, due to overlapping spectra in this region, while the dichlone method [12] measures at 580 nm but the molar absorptivity of LN–dichlone derivative is lower than that of LN–FDNB derivative. Additionally, the simultaneous spectrophotometric determination of LN and HCT at 205 and 225 nm, respectively, previously reported [15] may suffer from the concurrent absorption of excipients in the same region.

Although the procedure of the derivatization reaction in the proposed method takes 45 min, it can be applied in a great number of samples simultaneously, thus reducing the total analysis time. Finally, in comparison with other methods previously reported for the determination of LN in single or multicomponent formulations, the

Table 3
Assay results for the determination of LN in commercial tablets by the proposed and the official method

Formulation	Nominal content/tablet (mg)	% Found ± SD ($n = 3$)	% Found ± SD ($n = 5$)		% Relative difference		t-test[a]	
		Official method (HPLC)	Proposed method					
			356.5 nm	405.5 nm	356.5 nm	405.5 nm	356.5 nm	405.5 nm
Zestril	5	101.1 ± 1.3	99.1 ± 1.5	99.4 ± 2.1	−2.0	−1.7	1.908	1.245
	10	101.2 ± 1.2	99.8 ± 0.6	100.3 ± 1.0	−1.4	−0.9	2.256	1.152
	20	100.8 ± 0.8	101.4 ± 0.8	101.0 ± 0.9	0.6	0.2	1.027	0.315
Z-Bec	5	101.0 ± 1.4	98.8 ± 1.2	98.5 ± 1.8	−2.2	−2.5	2.373	2.039
	10	100.4 ± 0.5	99.5 ± 0.6	100.6 ± 0.9	−0.9	0.2	2.163	0.347
	20	100.5 ± 1.0	101.7 ± 0.7	100.3 ± 1.2	1.2	−0.2	2.030	0.240

[a] t, Theoretical, 2.447 (95% confidence level).

developed method is rapid, accurate, sensitive, overcomes the problem of low absorption of LN in the UV region and can be used for the routine analysis of LN in its single or multicomponent pharmaceutical preparations. In comparison with the official methods [19,20] the method developed is more rapid than potentiometric titration and of low cost in comparison with HPLC. The only disadvantage is that FDNB is a skin irritant [27] and must be handled with the appropriate precautions. Additionally the analytical methodology can be possibly further improved, offering automation, if combined with flow injection analysis [21]. The proposed method can also be applied for content uniformity, test and dissolution profile studies of LN formulations.

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