Use of ceric ammonium sulphate and two dyes, methyl orange and indigo carmine, in the determination of lansoprazole in pharmaceuticals

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Two spectrophotometric methods are proposed for the assay of lansoprazole (LPZ) in bulk drug and in dosage forms using ceric ammonium sulphate (CAS) and two dyes, methyl orange and indigo carmine, as reagents. The methods involve addition of a known excess of CAS to LPZ in acid medium, followed by determination of residual CAS by reacting with a fixed amount of either methyl orange, measuring the absorbance at 520 nm (method A), or indigo carmine, measuring the absorbance at 610 nm (method B). In both methods, the amount of CAS reacted corresponds to the amount of LPZ and the measured absorbance was found to increase linearly with the concentration of LPZ, which is corroborated by the correlation coefficients of 0.9979 and 0.9954 for methods A and B, respectively. The systems obey Beer's law for 0.5–7.0 µg mL^{-1} and 0.25–3.0 µg mL^{-1} for methods A and B, respectively. The apparent molar absorptivities were calculated to be 3.0 x 10^4 and 4.4 x 10^4 L mol^{-1} cm^{-1} for methods A and B, respectively. The limits of detection (*LOD*) and quantification (*LOQ*) were calculated to be 0.08 and 0.25 µg mL^{-1} for method A, and 0.09 and 0.27 µg mL^{-1} for method B, respectively. The intra-day and inter-day precision and accuracy of the methods were evaluated according to the current ICH guidelines. Both methods were of comparable accuracy ($e_r \leq 2$ %). Also, both methods are equally precise as shown by the relative standard deviation values < 1.5%. No interference was observed from common pharmaceutical adjuvants. The accuracy of the methods was further ascertained by performing recovery studies using the standard addition method. The methods were successfully applied to the assay of LPZ in capsule preparations and the results were statistically compared with those of the literature UV-spectrophotometric method by applying Student's *t*-test and *F*-test.

Keywords: lansoprazole, determination, spectrophotometry, ceric ammonium sulphate, methyl orange, indigo carmine, pharmaceuticals

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Lansoprazole (LPZ) is a substituted benzimidazole, chemically known as methyl--4-(2,2,2-trifluroethoxy)-2-pyridyl]methyl]sulfinyl]benzimidazole (Fig. 1). LPZ is a proton pump inhibitor (1), which inhibits the ultimate step in gastric acid secretion. Even the stimulus-independent acid secretion is suppressed. Both basal and stimulus acids are inhibited. Peptic activity is reduced secondary to acid inhibition. LPZ has a stronger inhibitory effect on *H. pylori* than omeprazole, and is therefore widely used in the treatment of benign gastric ulcer associated with *H. pylori*, duodenal ulcer and reflux oesophagitis. LPZ is also indicated for Zollinger-Ellison syndrome and acid related dyspepsia.

The therapeutic importance of LPZ justifies research aimed to develop analytical methods for its determination in body fluids and in pharmaceuticals. A high-performance thin-layer chromatographic (HPTLC) method for the detection and determination of LPZ in human plasma has been reported by Satin *et al.* (2). A sensitive quantitative method was developed for the estimation of reactive metabolite formation *in vitro*, the analysis being completed by HPLC coupled with a fluorescence detector and a mass spectrometer (3). However, no (or little) research has been done to determine LPZ in pharmaceuti-
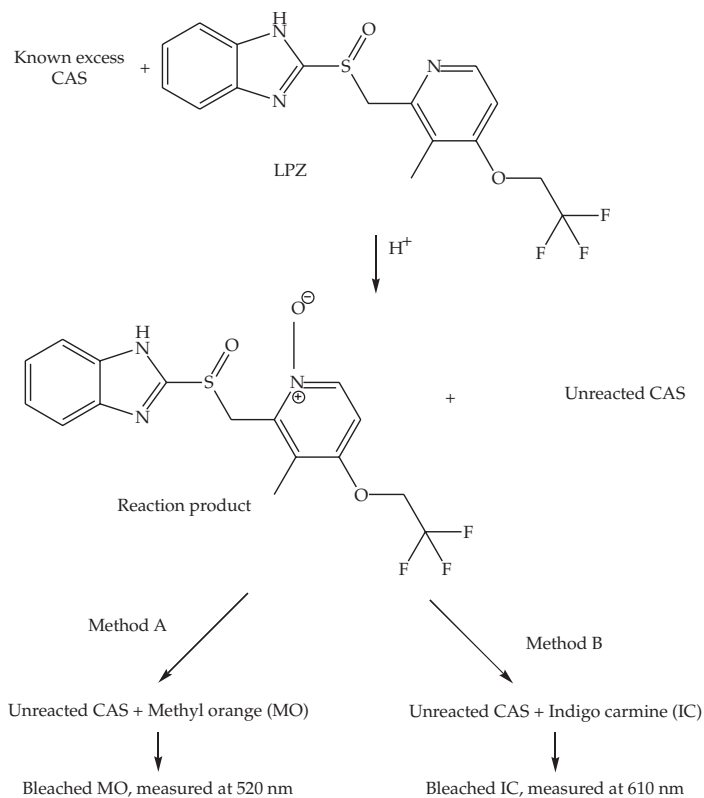

Fig. 1. Probable reaction scheme.

cals. There are two reports on the determination of LPZ in pharmaceuticals by HPLC (4, 5). In a recent communication, Yeniceli *et al.* (6) reported the UV-spectrophotometric determination of LPZ. The method is reported to be applicable in the range 2–20 µg mL^{-1} LPZ.

The only visible spectrophotometric method (7) reported is based on the formation of a blue chromogen measurable at 810 nm when LPZ was reacted with iron(III) chloride and ferricyanide in HCl medium. The present investigation aims to develop sensitive and cost-effective methods for the determination of LPZ in pure and in dosage forms using the spectrophotometric technique. The methods utilize ceric ammonium sulphate and methyl orange or indigo carmine as reagents.

EXPERIMENTAL

Apparatus

A Systronics model 106 digital spectrophotometer (Systronics India Ltd., India) with 1-cm matched quartz cells was used for all absorbance measurements.

Reagents and standards

All chemicals used were of analytical purity grade and all solutions were prepared in distilled water.

Ceric ammonium sulphate. – A 0.01 mol L^{-1} ceric ammonium sulphate (s.d. Fine Chem., India) solution was prepared in 1.0 mol L^{-1} sulphuric acid and standardized (8). This was diluted stepwise to obtain working concentrations containing 450 and 900 µg mL^{-1} for use in method A, and method B, respectively.

Methyl orange. – A 500 µg mL^{-1} dye solution was prepared by dissolving accurately weighed dye (s.d. Fine Chem., India, 85% dye content) in water, diluting it in a calibrated flask and filtering using glass wool. It was then diluted to obtain a working concentration of 50 µg mL^{-1}.

Indigo carmine. – A 1000 µg mL^{-1} stock standard solution was prepared by dissolving accurately weighed dye (s.d. Fine Chem, 90% dye content) in water and diluting it to volume in a calibrated flask. The solution was then diluted to get a working concentration of 200 µg mL^{-1}.

Standard solution of lansoprazole. – Pharmaceutical grade lansoprazole was received as a gift from Cipla Ltd, India; it was reported to be 99.8% pure and was used as received. A stock standard solution equivalent to 200 µg mL^{-1} LPZ was prepared by dissolving an accurately weighed amount of pure drug in 1 mol L^{-1} hydrochloric acid and diluting it with the same acid to a known volume; it was later diluted appropriately with water to get working concentrations of 20 and 10 µg mL^{-1} for use in spectrophotometric methods A and B, respectively. The standard solutions were kept in amber coloured bottles and stored in a refrigerator when not in use.

Procedures

Method A. – Different aliquots (0.25–3.5 mL) of the standard 20 µg mL^{-1} LPZ solution were transferred into a series of 10-mL calibrated flasks by means of a micro burette and the total volume was adjusted to 4 mL by adding an adequate quantity of water. To each flask, 1 mL each of 5 mol L^{-1} HCl and CAS solution (450 µg mL^{-1}) were added, the later measured accurately. The content was mixed and let stand for 15 min with occasional shaking. Finally, 1 mL of 50 µg mL^{-1} methyl orange solution was added (accurately measured) and the volume was diluted to the mark with water and mixed well. The absorbance of each solution was measured at 520 nm against a reagent blank after 5 min.

Method B. – Varying aliquots (0.25–3.0 mL) of the standard 10 µg mL^{-1} LPZ solution were transferred into a series of 10-mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. To each flask, 1 mL each of 5 mol L^{-1} hydrochloric acid and CAS solution (900 µg mL^{-1}) were added by means of a micro burette. The content was mixed well and the flasks were kept aside for 15 min with intermittent shaking. Finally, 1 mL of 200 µg mL^{-1} indigo carmine solution was added to each flask, the volume was diluted to the mark with water, mixed well and absorbance was measured against a reagent blank at 610 nm after 5 min.

In both methods, a standard graph was prepared by plotting the absorbance *vs.* the concentration of LPZ. The unknown concentration was read from the calibration graph or computed from the regression equation derived using Beer's law data.

Procedure for capsules. – The following formulations containing LPZ were purchased from local commercial sources and used in the investigation: Lanzol (Cipla India Ltd.) containing 15/30 mg of LPZ and Lanzopen (Morepen Labs, India) containing 15/30 mg of LPZ per capsule.

A quantity of the capsule powder equivalent to 20 mg of LPZ was accurately weighed into a 100-mL calibrated flask, 60 mL of 1 mol L^{-1} HCl was added and the content was shaken for 20 min; the volume was finally diluted to the mark with 1 mol L^{-1} HCl, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 mL portion of the filtrate was discarded and the filtrate (200 µg mL^{-1} LPZ) was appropriately diluted with water to get 20 and 10 µg mL^{-1} LPZ and analyzed by taking convenient aliquots (1 or 2 mL) according to the procedures described earlier.

<center>RESULTS AND DISCUSSION</center>

Method development

The proposed spectrophotometric methods are indirect and are based on determination of the residual CAS after bringing the reaction between LPZ and CAS to completion. The residual CAS was determined by reacting with a fixed amount of either methyl orange or indigo carmine dye.

When added in increasing concentrations to a fixed concentration of CAS, LPZ consumes the latter proportionally and there is a concomitant drop in the remaining concen-

tration of CAS. When a fixed dye concentration is added to decreasing concentrations of CAS, a concomitant increase in the dye concentration results. Consequently, a proportional increase in absorbance at the respective λ_{max} is observed with increasing concentration of LPZ.

Preliminary experiments were performed to fix the upper concentrations of the dyes that could be determined spectrophotometrically, and these were found to be 5 and 20 µg mL^{-1} for methyl orange and indigo carmine, respectively. A CAS concentration of 45 µg mL^{-1} was found to bleach the red colour due to 5 µg mL^{-1} methyl orange, whereas 90 µg mL^{-1} CAS was required to destroy the blue colour due to 20 µg mL^{-1} indigo carmine. Hence, different concentrations of LPZ were reacted with 1.0 mL of 450 µg mL^{-1} CAS in method A and 1.0 mL of 900 µg mL^{-1} CAS in method B, followed by determination of residual CAS as described under the respective procedure.

For both steps, *i.e.*, the reaction between LPZ and CAS, and the determination of the latter by reacting with the dye, HCl medium (5 mol L^{-1}) was found to be ideally suited. One mL of 5 mol L^{-1} acid in a total volume of about 3–4 mL was used for the first step, and the same quantity of acid was maintained for the bleaching step. Reaction time of 15 min is not critical and any delay up to 30 min did not affect the absorbance reading. The absorbance of both dye colours was constant for several hours even in the presence of the reaction product.

Analytical data

A linear correlation was found between absorbance at λ_{max} and LPZ concentration. The graph showed negligible intercept:

$$A = 0.010 + 0.079 \, \gamma; \; R = 0.9979, \; n = 8 \; \text{(method A)}$$

$$A = 0.006 + 0.117 \, \gamma; \; R = 0.9954, \; n = 7 \; \text{(method B)}$$

where A is the absorbance and γ is concentration in µg mL^{-1}, R is the regression coefficient and n is the number of concentration levels.

Table I. Analytical and regression parameters of proposed methods

Parameter	Method A	Method B
λ_{max} (nm)	520	610
Beer's law limits (µg mL^{-1})	0.5–7.0	0.25–3.0
Molar absorptivity (L mol^{-1} cm^{-1})	3.0×10^4	4.4×10^4
Limit of detection (µg mL^{-1})	0.08	0.09
Limit of quantification (µg mL^{-1})	0.25	0.27
Regression equation Intercept: a ± S$_a$ Slope : b ± S$_b$ (γ, LPZ µg mL^{-1})	0.010 ± 0.012 0.079 ± 0.002	0.006 ± 0.012 0.117 ± 0.006
Correlation coefficient, R	0.9979	0.9954

The optical characteristics such as Beer's law limits and molar absorptivity values for both methods are given in Table I. The limits of detection (*LOD*) and quantification (*LOQ*) calculated according to ICH guidelines (9) as the ratio of 3.3 and 10 standard deviations of the blank ($n = 7$), and the slope of the calibration line.

Performance characteristics

To evaluate the accuracy and intra-day precision of the methods, a pure drug solution was analyzed at three different levels (concentrations), each determination being repeated seven times. The relative error (%) and relative standard deviation (%) values are summarized in Table II. From Table II, it is clear that method A with a relative error of < 1.5% is as accurate as method B with 1.2–2%. Moreover, both methods are found to be equally precise with RSD values in the range of 1.3–1.9% for method A and 1.3–1.7% for method B. For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed, in which the standard drug solution was determined at three different levels each day for five days, with all solutions being prepared afresh each day. The day-to-day relative standard deviation values were in the range of 0.6–3.3% and represent the best appraisal of repeatability of the proposed methods.

Table II. Accuracy and precision

Method	LPZ taken ($\mu g\ mL^{-1}$)	e_r (%)[a]	RSD (%)	CL[b]
A	2.00	1.1	1.5	1.98 ± 0.03
	4.00	1.3	1.9	3.95 ± 0.07
	6.00	1.3	1.3	5.92 ± 0.07
B	0.50	1.2	1.5	0.49 ± 0.01
	1.50	2.0	1.3	1.47 ± 0.02
	2.50	1.3	1.7	2.47 ± 0.04

CL – confidence limits.
[a] Mean value of seven determinations.
[b] At 95% confidence level for 6 degrees of freedom.

As a part of selectivity studies, the accuracy and validity of the proposed methods were further ascertained by performing recovery experiments. Pre-analyzed capsule powder was spiked with pure LPZ at three different levels and the total was found by the proposed methods. Each determination was repeated three times. The drug recovery was found to vary from 97.7–104.5% with RSD of 1.1–2.1% for method A, and for method B the recovery was in the range 98.6–105.3% with RSD values in the range 0.9–2.1% (Table IV). This revealed that co-formulated substances such as talc, starch, gelatin, gum acacia, calcium carbonate, calcium gluconate, calcium dihydrogen orthophosphate, sodium alginate and magnesium stearate did not interfere with the determination.

Table III. Determination of lansoprazole in formulations and statistical comparison with the literature method

Capsule brand name	Nominal amount (mg)	Found (%) $\quad$ SD[a]		
		Literature method (6)	Method A	Method B
Lanzol	15	97.1 ± 1.1	99.4 ± 1.7 $t = 2.65$ $F = 2.51$	98.2 ± 1.9 $t = 1.17$ $F = 3.28$
	30	101.2 ± 0.8	99.8 ± 1.1 $t = 2.37$ $F = 2.23$	102.2 ± 1.1 $t = 1.76$ $F = 1.96$
Lanzopen	15	100.5 ± 1.2	102.8 ± 1.6 $t = 2.57$ $F = 1.74$	98.6 ± 1.8 $t = 2.01$ $F = 2.08$
	30	101.5 ± 0.9	99.8 ± 1.3 $t = 2.45$ $F = 2.30$	102.2 ± 1.3 $t = 1.01$ $F = 2.34$

[a] Mean value of five determinations.
Tabulated *t*-value at 95% confidence level is 2.77; tabulated *F*-value at 95% confidence level is 6.39.

A separate selectivity test was performed by applying the proposed methods to the determination of LPZ in a synthetic mixture consisting of LPZ (100 mg), talc (250 mg), starch (300 mg), lactose (30 mg), calcium gluconate (50 mg), calcium dihydrogenorthophosphate (20 mg), sodium alginate (70 mg) and magnesium stearate (100 mg), in the ratio of 1:2.5:3.0:0.3:0.5:0.2:0.7:1. LPZ was extracted with three 20 mL portions of water and filtered. The filtrate was washed with water; the filtrate and washings were collected in a 100 mL-calibrated flask and diluted to volume with water and mixed well. A convenient aliquot of the extract was subjected to analysis. The recovery (%) of LPZ from placebo was found to be 102.5 ± 1.3 for method A and 101.3 ± 1.2 for method B.

Application

Table III gives the results of the assay and reveals that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a literature UV-spectrophotometric method (6) by applying Student's *t*-test for accuracy and *F*-test for precision. The literature method was based on the measurement of the absorbance of the drug solution in 0.01 mol L^{-1} NaOH at 292 nm. At the 95% confidence level, the calculated *t*- and *F*-values did not exceed the tabulated values (*t* = 2.77 and *F* = 6.39), suggesting that the proposed methods are as accurate and precise as the literature method.

They rely on the use of simple and cheap chemicals, and inexpensive techniques, but provide a sensitivity comparable to that achieved by sophisticated and expensive techniques like HPLC (Table V).

Table IV. Recovery experiments using the standard addition method

Formulation studied	Method A			Method B		
	Drug in tablet (μg mL^{-1})	Pure drug added (μg mL^{-1})	Drug recovered[a] (mean ± SD) (%)[a]	Drug in tablet (μg mL^{-1})	Pure drug added (μg mL^{-1})	Drug recovered[a] (mean ± SD) (%)[a]
Lanzol 15	1.99	1.00	98.6 ± 1.1	0.98	0.50	100.5 ± 1.2
	1.99	3.00	103.5 ± 1.5	0.98	1.00	101.6 ± 1.3
	1.99	5.00	100.3 ± 1.9	0.98	1.50	98.6 ± 1.5
Lanzopen 30	2.00	1.00	99.3 ± 2.1	1.02	0.50	99.6 ± 2.1
	2.00	3.00	97.7 ± 1.4	1.02	1.00	105.3 ± 1.0
	2.00	5.00	104.5 ± 1.1	1.02	1.50	101.3 ± 1.9

$n = 3$

Table V. Comparison of the proposed and reported methods

Reported methods	Reagents used	λ_{max}, nm	Range (μg mL^{-1})	Remarks	Ref.
HPTLC	–	–	0.05–0.25	Narrow linear dynamic range	2
HPLC-MS	–	–	–	Highly expensive instrumental set up required	3
HPLC	–	254	–	pH has to be carefully maintained, expensive instrumental set up	4
HPLC	–	254	0.3–60	pH has to be carefully maintained, expensive instrumental set up	5
UV-spectrophotometry	0.01 mol L^{-1} NaOH	292	2–20	–	6
Vis-spectrophotometry	Iron(III) chloride- -ferriccyanide	810	–	–	7
Vis-spectrophotometry	A – CAS-methyl orange	520	0.5–7.0 ($\varepsilon = 3.0 \times 10^4$)	Highly sensitive, wide linear dynamic ranges, inexpensive instrumental setup, use of eco-friendly chemicals	This paper
	B – CAS-indigo carmine	610	0.25–3.0 ($\varepsilon = 4.4 \times 10^4$)		

ε – molar absorptivity (L mol^{-1} cm^{-1}).
CAS – ceric ammonium sulphate.

CONCLUSIONS

Two useful methods for the determination of LPZ have been developed and validated as per the current ICH guidelines. The proposed methods are simple, rapid and use CAS solution as a quantitative reagent, highly stable in solution, and are based on the measurement of stable coloured species. The proposed methods do not take more than 15–20 min and are among the most sensitive ever reported for lansoprazole. These advantages coupled with good accuracy and precision make the methods highly suitable for routine use in pharmaceutical laboratories as a part of industrial quality control.

REFERENCES

1. *Current Index of Medical Specialities (CIMS), Updated Prescriber's Hand Book*, CMP Medica India Pvt Ltd., Bangalore 2005, p. 72.
2. M. C. Satia, I. A. Modi, R. I. Modi, B. K. Chakravarthy and T. P. Gandhi, High-performance thin-layer-chromatographic method for the detection and determination of lansoprazole in human plasma and its use in pharmacokinetic studies, *J. Chromatogr. B: Biomed. Sci. Appl.* **693** (1997) 199–204.
3. J. Gan, T. W. Harper, M. M. Hsuch, Q. Qu and W. G. Humphreys, Dansyl glutathione as a trapping agent for the quantitative estimation and identification of reactive metabolites, *Chem. Res. Toxicol.* **18** (2005**)** 896–903.
4. N. Badwe, G. C. Kandapal and S. T. Hathiari, Estimation of lansoprazole in pharmaceutical dosage forms using HPLC, *Eastern Pharm.* **39** (1996) 127–128.
5. D. G. Sankar, K. D. Priya, M. V. Krishna and P. V. M. Latha, Estimation of lansoprazole in pure form and in pharmaceutical dosage forms by RP-HPLC, *Int. J. Chem. Sci.* **3** (2005) 247–252.
6. D. Yeniceli, D. Dogaukol-Ak and M. Tuncel, Determination of lansoprazole in pharmaceutical capsules by flow injection analysis using UV-detection, *J. Pharm. Biomed. Anal.* **36** (2004) 145–148.
7. A. Ramesh, K. E. Kumar, M. Sudhakar and B. K. Murthy, Spectrophotometric method for the estimation of lansoprazole in pharmaceutical formulations, *Int. J. Chem. Sci.* **2** (2004) 642–644.
8. J. Bassett, R. C. Denney, G. H. Jefferey and J. Mendham, *Vogel's Quantitative Inorganic Analysis*, 4th ed., English Language Book Society and Longman, London 1978, p. 366.
9. ICH Harmonized Tripartite Guideline prepared within the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) Q2B, *Validation of Analytical Procedures: Methodology*, ICH, London 1996.

S A Ž E T A K

Uporaba cerijevog amonijevog sulfata i dviju boja, metiloranža i indigo karmina, u određivanju lansoprazola u farmaceutskim pripravcima

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Predložene su dvije spektrofotometrijske metode za određivanje lansoprazola (LPZ) kao čiste supstancije i u doziranim ljekovitim pripravcima koristeći cerijev amonijev sulfat (CAS) i dvije boje, metiloranž i indigo karmin. Metode uključuju dodatak suviška CAS-a u otopinu LPZ u kiselom mediju, nakon čega višak reagensa reagira s poznatom količinom metiloranža (metoda A, mjerenje apsorbancije na 520 nm) ili indigo karminom (metoda B, mjerenje apsorbancije na 610 nm). U obje metode, količina CAS-a koja reagira odgovara količini LPZ i izmjerena apsorbancija linearno ovisi o koncentraciji LPZ-a, uz koeficijent korelacije 0,9979 i 0,9954 za metodu A odnosno B. Oba sustava slijede Beerov zakon u koncentracijskom rasponu 0,5–7,0, odnosno 0,25–3,0 μg mL^{-1}. Molarni apsorpcijski koeficijent određen metodom A iznosio je $3,0 \times 10^4$ a metodom B $4,4 \times 10^4$ L mol^{-1} cm^{-1}. Granica detekcije (*LOD*) i kvantifikacije (*LOQ*) bile su 0,08 i 0,25 μg mL^{-1} za metodu A, te 0,09 i 0,27 μg mL^{-1} za metodu B. Preciznost i ispravnost metoda procijenjena je prema važećim ICH smjernicama. Obje metode su podjednako ispravne ($e_r \leq 2\%$) i precizne (RSD < 1,5%). Nije primijećena interferencija s uobičajenim pomoćnim tvarima. Ispravnost je procijenjena i metodom standardne adicije. Rezultati su statistički uspoređeni s referentnom UV-spektrofotometrijskom metodom pomoću Studentovog *t*-testa i *F*-testa.

Ključne riječi: lansoprazol, određivanje, spektrofotometrija, cerijev amonijev sulfat, metiloranž, indigo karmin, ljekoviti pripravci

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