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Short Communication

Liquid chromatographic determination of indapamide in the presence of its degradation products

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Introduction

Indapamide is the first of the new class of antihypertensive/diuretics, the indolines. It is indicated in the treatment of hypertension alone or in combination with other antihypertensives. It is also prescribed in the treatment of salt and fluid retention associated with congestive heart failure [1]. Indapamide is currently being marketed only in a tablet dosage form, but has the potential for other dosage forms like liquids, semisolids, etc.

For the development of indapamide in liquid/semisolid dosage forms, it is imperative that the parameters involved in its degradation are well understood. In order to study this, one requires a suitable stability-indicating method of analysis; i.e. a method capable of detecting the pure drug in the presence of its degradation products.

Three methods of analysis for indapamide are reported in the literature [2–4]. One of these methods is a fluorometric analysis which involves heating indapamide with alkali at 100°C for 1 h, treatment with formaldehyde and measuring the flourescence [2]. The other two methods involve the use of reversed-phase liquid chromatography (RP–LC). Of the two RP–LC methods one uses a μBondapak C-18 column and methanol–water–acetic acid–ethansulphonic acid sodium salt as mobile phase [3], and the other uses a Lichrosorb C-18 column with acetonitrile–sodium acetate buffer pH 3.6 as mobile phase [4]. None of the methods are reported to be stability-indicating, and the sample preparation for all these methods is laborious and time consuming. Therefore, there was a need to develop a rapid and simple stability-indicating method of analysis for indapamide to study its solution stability.

Experimental

Reagents

All reagent grade chemicals and HPLC grade solvents were obtained from Fisher Scientific (Fairlawn, NJ, USA). Indapamide was obtained from Labochim (Italy). Triethylamine 99% gold label and 4-chloro-3-sulphamoyl benzoic acid were obtained from Aldrich (Milwaukee, WI, USA).

Instrumentation

The LC system consisted of a Waters model 6000A LC pump, WISP model 710 automatic injector and model 450 variable wavelength detector (Waters, Milford, MA, USA), and a Hewlett–Packard model 3390A integrator–plotter (Hewlett–Packard, Avondale, PA, USA). The HPLC column was a chemically bonded octadecylsilane column (Lichrosorb RP-18, 5 μ, 250 × 4.6 mm i.d., Alltech, Deerfield, IL, USA).

Mobile phase

The mobile phase consisted of methanol–aqueous acetic acid (1%) with triethylamine

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(0.2%) (50:50, v/v). The mobile phase was filtered after mixing through MSI cellulosic 0.45 μ filters (MSI, Westboro, MA, USA). The mobile phase was degassed using vacuum (10 psi) for 10 min.

Chromatographic conditions

The column temperature was ambient (24–26°C) and the detector was set at 250 nm. This wavelength was chosen, since it gave the highest selectivity for the drug with maximum freedom from interference [5]. The sensitivity of the detector was set at 0.02. The integrator was set at an attenuation of 4 and threshold of 4, with a chart speed of 0.3 cm min^{-1}. The injection volume was 20 μl and the mobile phase flow rate was 1.0 ml min^{-1}.

Preparation of standard and assay solutions

A stock solution of indapamide was prepared in methanol (HPLC grade) to give a concentration of 1.0 mg ml^{-1}. This solution was then diluted with mobile phase to give standard solutions with concentrations of 1, 2, 5, 10, 20 and 50 μg ml^{-1}. The standard solutions were injected in duplicate and the detector response was measured as peak area using an integrator–plotter. The peak area was regressed versus concentration to obtain a calibration plot for the standard indapamide solutions.

Sample solutions of indapamide for analysis were prepared by diluting 1.0 ml of the stock solution in a 100 ml volumetric flask to volume with the mobile phase. These samples were freshly prepared and injected immediately after preparation.

Degradation of indapamide

Indapamide was degraded with acid (0.01 N HCl) and alkali (0.01 N NaOH) at 55°C for 6 months [6]. Samples of 1.0 ml were withdrawn and diluted to 100 ml with the mobile phase and were run using the LC method developed in our laboratory.

Validation

The LC method developed, was validated with respect to the following criteria: non-interference of peaks, sensitivity, linearity of response, tailing factor, and precision.

Linearity of response was studied by running a standard curve of indapamide. Six standard solutions, namely 1, 2, 5, 10, 20 and 50 μg ml^{-1} were injected to check for linearity. For determining sensitivity of detection, dilutions of stock indapamide solution were made till no response/drug peak was observed in the HPLC run. The tailing factor (T) was calculated using the following equation [7]:

$$T = W_{0.05}/(2f) \quad (1)$$

where T = tailing factor, $W_{0.05}$ = width of the peak at 5% height, and f = the distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline. Method precision studies were carried out by injecting 10 replicate injections of standard indapamide solution from the same vial.

Results and Discussion

Figure 1 shows the chromatograms obtained for the standard solution of indapamide, product mixture of completed degraded indapamide and degradation products spiked with indapamide solution, with a Lichrosorb RP-18 column and mobile phase as previously described. Figure 1 shows that indapamide eluted in 11.00 min and was well separated from its degradation products.

The method was validated to be stability-indicating by chromatographing indapamide with its degradation products. One of the major degradation products, 4-chloro-3-sulphamoyl benzoic acid (CSBA) was obtained commercially. Standard samples of CSBA were spiked with standard samples of indapamide and injected. CSBA eluted at 3.50 min which also was the retention time for one of the two degradation product peaks in the degraded indapamide samples. This showed that indapamide undergoes amide hydrolysis under acidic as well as basic conditions (Fig. 2) and breaks down into CSBA and N-amino-2-methyl indoline (NAMI). This also showed that the two major degradation product peaks of indapamide are well separated and do not interfere with the drug peak.

The standard plot of indapamide showed a linearity of response with a correlation coefficient (R^2) of 0.9999. The method was able to detect indapamide in concentrations as low as 0.5 μg ml^{-1}. The tailing factor study showed a factor of 1.12 for indapamide peak. Method precision studies were carried out by injecting 10 replicate injections from the same vial and calculating standard deviation and relative standard deviation (RSD) for the peak areas

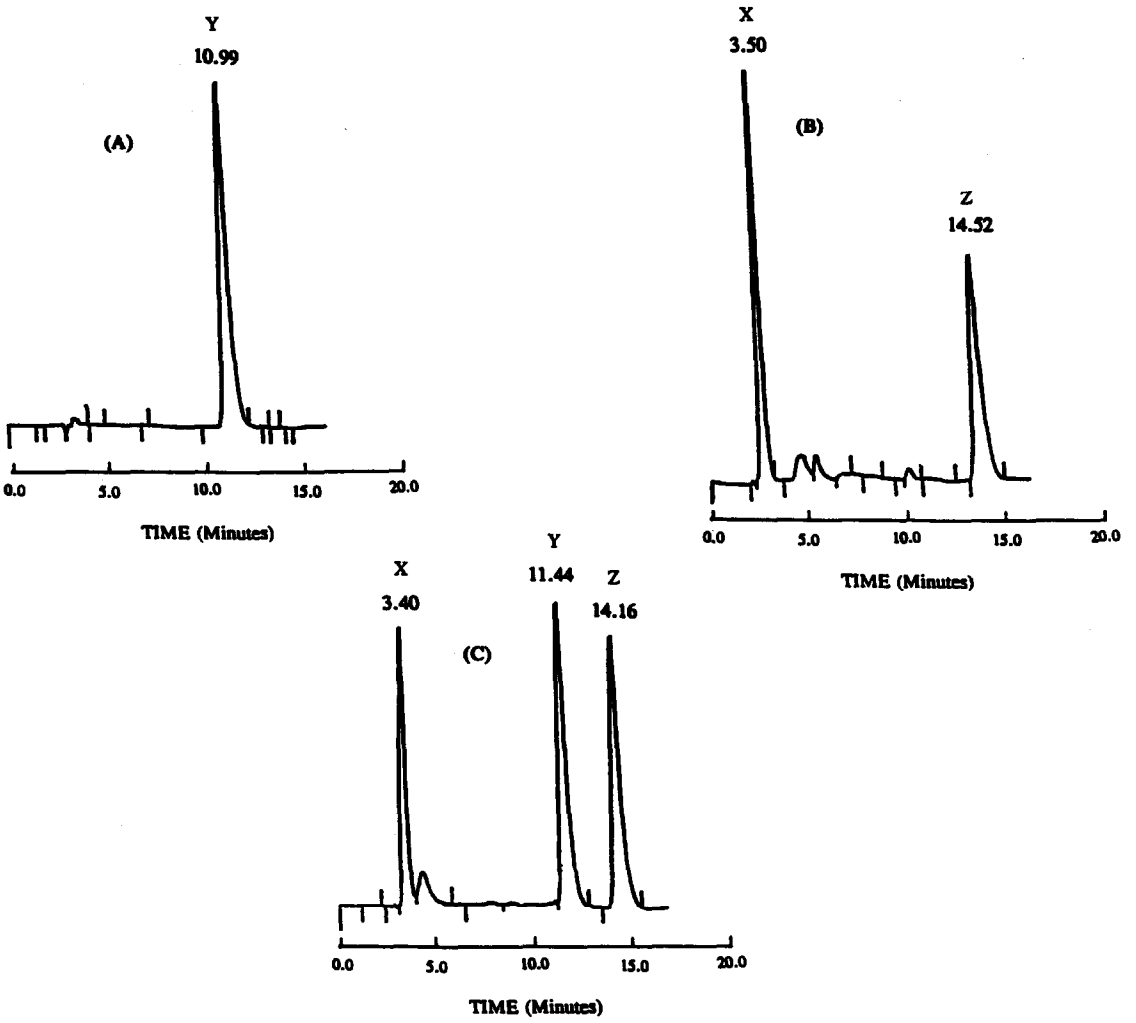

Figure 1
Representative chromatograms for indapamide standard (A), completely degraded indapamide (B), and indapamide spiked with degradation product mixture (C), with a Licrosorb C-18 column and mobile phase as described in the Experimental section. X, NAMI; Y, indapamide; and Z, CSBA.

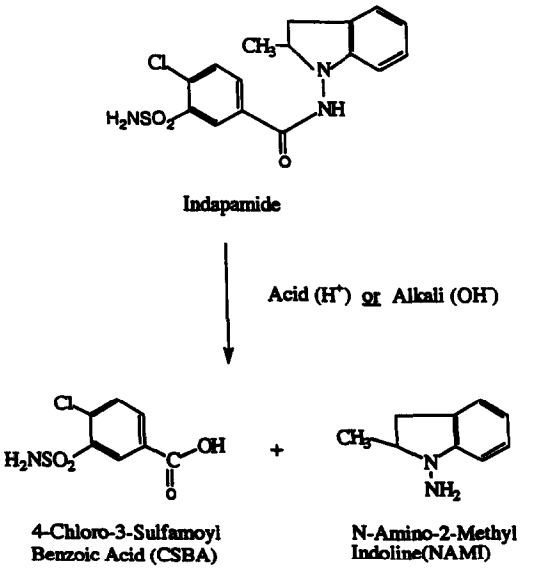

Figure 2
Hydrolytic degradation of indapamide.

obtained. An RSD of 1.21% was observed for the 10 samples. The low RSD values observed indicate that the method is precise.

The method was used to study degradation kinetics of indapamide solutions. The degradation was found to be pH-dependent and the pH–rate profile was biphasic. Maximum stability of the drug was observed at pH 5.0 and 9.5, with rate of degradation significantly increasing below pH 5.0, between pH 6 and 9 and above pH 9.5. The degradation showed weak temperature dependency at both pH 5.0 and 9.5. No significant effect of light or dissolved air was observed on the degradation of indapamide.

This method allows for simple and rapid analysis of indapamide solutions. A study carried out also showed that the presence of buffers (phosphate, acetate, glycine) and co-solvents (PEG, propylene glycol, ethanol) did not interfere with the assay, and therefore the method is suitable as a stability-indicating method for the measurement of indapamide stability in pharmaceutical preparations.

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