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Simultaneous determination of aspirin and isosorbide 5-mononitrate in formulation by reversed phase high pressure liquid chromatography

M. Gandhimathi [1,*], T.K. Ravi, Annie Abraham, Renu Thomas

Department of Pharmaceutical Analysis, College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, 395, Sarojini Naidu Street, Coimbatore 641 044, Tamil Nadu, India

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Abstract

A simple, precise and rapid reversed phase HPLC method was developed for the simultaneous estimation of aspirin (AS) and isosorbide 5-mononitrate (ISM) in combined formulation. The method was carried out on a Thermo Quest C18 column using a mixture of water:methanol (water pH adjusted to 3.4 using dilute orthophosphoric acid) and detection was carried out at 215 nm using chlorzoxazone as internal standard. Both the drugs showed linearity in the range of 2–10 μg/ml and limits of quantification was found to be 4 and 40 ng/ml for AS and ISM, respectively.

Keywords: Aspirin; Isosorbide; Simultaneous estimation

1. Introduction

Aspirin, [1,2] (AS) chemically known as acetyl salicylic acid and is used as non-steroidal anti-inflammatory and analgesic drug. Isosorbide 5-mononitrate (ISM) chemically known as 1, 4:3, 6-dianhydro-D-glucitol 5-mononitrate which is used as antianginal drug. A combination of 75 mg of AS and 60 mg of ISM is commercially available in tablet form.

Estimation of AS from human plasma and urine was reported by Harrison et al. [3] and Kess et al. [4]. These methods were carried out using ODS columns and by UV detection. HPLC methods for the estimation of AS from pharmaceutical dosage form were reported by Fogel et al. [5] and Galante et al. [6].

Determination of ISM and its metabolites from human plasma by HPLC method was developed [7]. Two validated HPLC methods [8,9] were developed for the estimation of ISM in formulation. Synthesis, hydrolytic kinetics and antiplatelet effects of ISM derivatives of AS was carried out by Gilmer et al. [10]. The reported methods are applicable for the estimation of either for AS or

* Corresponding author. Tel.: +91-422-210075.
E-mail address: gands72@yahoo.co.in (M. Gandhimathi).
[1] Tel.: +91-4259-221413 (Resident).

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ISM individually from pharmaceutical dosage forms or biological fluids.

No single method was reported for the estimation in combined dosage form. The present work describes the development of a validated analytical RP-HPLC method, which can quantify these components simultaneously from a combined dosage form.

2. Experimental

2.1. Reagents and chemicals

AS and ISM were received as gift samples from Torrent Pharmaceuticals Limited, India. HPLC grade solvents were purchased from S.D. Fine Chemicals Limited, India. Analytical grade chemicals were obtained from Qualigens Fine Chemicals, India.

2.2. Apparatus

HPLC was carried out using a model LC-10A system (Shimadzu, Japan) equipped with an SPD10A UV-Visible detector. The column used was Thermo Quest C_{18}, (150 × 4.6 mm I.D. 5 μm).

2.3. HPLC conditions

A mixture of water:methanol (water pH adjusted to 3.4 using dilute orthophosphoric acid) (60:40 v/v) was used as mobile phase at a flow rate of 1 ml/min with an operating pressure of 3000 psi. A Rheodyne® 7725i injector with a 20 μl loop was used for the injection of samples. Detection was done at 215 nm, with a sensitivity of 0.001 AUFS. The mobile phase was filtered through 0.2 μm membrane filter and degassed. The separation was carried out at room temperature, 25 ± 1 °C.

2.4. Preparation of standard solutions

The stock solutions of AS and ISM each of 1 mg/ml concentration in mobile phase and IS (chlorzoxazone, 250 μg/ml) in mobile phase were prepared and used. The working standards of AS and ISM were prepared by dilution of standard solution with mobile phase. For the working solution of internal standard 1 ml of stock solution of I.S was diluted to 10 ml with mobile phase when required.

2.5. Analysis of formulation

Twenty tablets, each containing 75 mg of AS and 60 mg of ISM were weighed and finely powdered. A quantity of powder equivalent to 10 mg of AS and 12.5 mg of ISM was weighed accurately and transferred to a 25 ml volumetric flask and the volume was made up with the mobile phase and it was filtered using 0.2 μm membrane filter. From the above prepared solution, 1 ml is taken and diluted to 10 ml with the mobile phase. From this 1 ml aliquot was mixed with 1 ml of internal standard solution (which contains 25 μg/ml of chlorzoxazone) and made up to 10 ml in a standard flask with the mobile phase. The 20 μl of the above solution is injected in to the column and chromatogram was recorded.

2.6. Recovery studies

Recovery studies were carried out by adding 10 mg of standards of AS and ISM to an equivalent quantity of the formulation, and 2.5 ml of internal standard (25 μg/ml) was added in a 25 ml volumetric flask. The volume was made up with mobile phase. Further dilutions were made and the contents of AS and ISM were once again determined by the proposed method by recording the chromatograms. The concentration of AS and ISM added, amount found after recovery study, the % R.S.D. and % accuracy are shown in Table 1. From the amount of the drug present, percentage recovery was calculated and shown in Table 2.

3. Results and discussion

Chromatograms of mixed standard solutions which contained AS and ISM along with internal standard (chlorzoxazone) were recorded and shown in Fig. 1. The retention times of ISM, AS and I.S were 2.4, 4.7 and 10.8 min, respectively.

Table 1
Validation of the analytical method

Concentration added (μg/ml)		Concentration found (μg/ml)		R.S.D. %		Accuracy %	
AS	ISM	AS	ISM	AS	ISM	AS	ISM
Intra assay ($n=6$)							
50	50	49.6	49.1	0.07	0.1	−0.8	−1.6
100	100	101.4	99.4	0.04	0.2	0.72	−0.6
150	150	148.0	150.3	0.05	0.4	−0.99	0.24
Inter assay ($n=12$)							
50	50	48.1	49.5	0.01	0.3	−4.0	−2.0
100	100	99.7	100.7	0.12	0.2	−0.3	0.7
150	150	151.7	149.2	0.03	0.1	1.1	−0.91

Calibration curves were obtained by plotting peak area ratios of standard of AS and ISM to internal standard versus concentration. AS and ISM showed linearity in the range of 2–10 μg/ml. The slope and intercept values were found to be 0.0278 and 0.2474 for AS and 0.0001 and 0.0566 for ISM, respectively. The percentage R.S.D. of slope and intercept value for AS was 0.25 and 0.15 and for ISM 0.73 and 0.31, respectively. The correlation coefficient values were found to be 0.9999 and 0.9992 for AS and isosorbide, respectively.

From the marketed formulation, sample solutions were made and spiked with internal standard (chlorzoxazone), and the response factor was used to calculate the concentration of each drug. From the concentration, the amounts of each drug present in tablets were calculated (Table 2).

The limits of quantification (LOQ) for AS and ISM were determined by injecting subsequently diluted solutions of AS and ISM and was found to

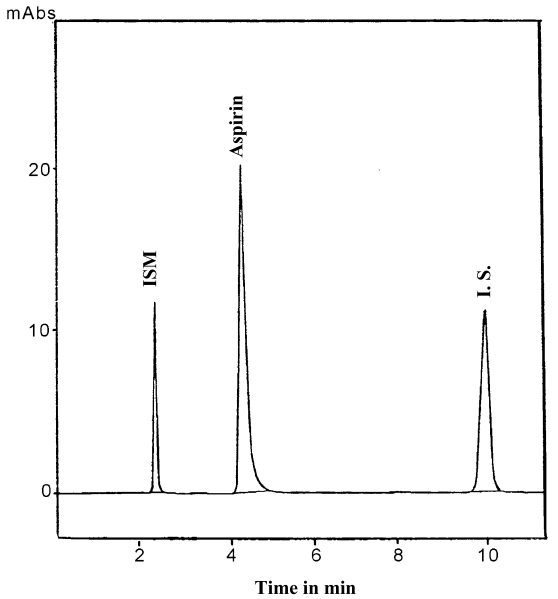

Fig. 1. Chromatogram of AS and ISM spiked with internal standard (2.5 μg/ml).

Table 2
Analysis of formulations and recovery studies by RP-HPLC

Drug	Amount (mg/tablet)		% Label claim[a]	% recovery[a]
	Labelled	Found[a]		
Isosorbide 5-mononitrate	60	59.89±1.76	99.81±1.32	99.27±0.56
Aspirin	75	74.17±1.57	98.87±0.97	99.53±0.81

[a] Mean±S.D. of six observations.

be 4 and 40 ng/ml, respectively. The minimum detectable quantity was 100 pg/ml for AS and 10 ng/ml for ISM. The LOQ values prove that this method can be effectively used for estimating AS and ISM drugs in biological samples also.

Precision of the method was studied by making repeated injections of the mixture of drugs. The coefficient variation (CV) after five determination was 1.54% at 8 ng/ml of AS and 1.16% at 50 ng/ml of ISM. The stability of analytes (AS and ISM) during analysis was observed in room temperature and under refrigeration. This was carried out by injecting the stored samples and by comparing the peak areas of AS and ISM with the peak areas of freshly prepared solutions of AS and ISM. They were stable up to 5 h under room temperature and for 3 days under refrigeration.

The amount obtained by this method was compared with methods reported [5,8] for the individual estimation of these two drugs. The percentage label claim obtained by proposed method was in good agreement for both the drugs individually and also in combination. A placebo prepared using lactose, carboxy methylcellulose and starch was used to study the effect of excipients on quantification of AS and ISM. There was no interference found due to excipients was supported by the % R.S.D. values of recovery studies (0.057 for AS and 0.22 for ISM). Further this method eliminates complicated extraction of individual drugs for quantitation. Both the drugs are estimated with in 15 min. Hence the present method is less cost effective and faster analytical method. The application of suggested procedure was successfully applied to the detection of these drugs in pharmaceutical preparation with high % recovery, good accuracy and precision. Hence this method is simple, sensitive and readily adaptable to routine determination of AS and ISM in combined formulation.

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