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Full Length Article

Spectrophotometic determination of Lorazepam in pharmaceutical tablets using batch and reverse flow injection methods

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ABSTRACT

A reverse flow injection analysis (RFIA) spectrophotometric method have been developed and compared with batch method for the determination of lorazepam (LOR) at the microgram level in pure and pharmaceutical dosage forms. The methods are based on the reaction of LOR with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in the presence of ferric chloride in aqueous medium. The water soluble green color product was measured at λ_{max} 659 nm. Under the optimum conditions, linearity was observed from 2 to 40 and 25 to 400 μg/mL LOR with detection limits of 0.61 and 2.29 μg/mL LOR by batch and RFIA procedures respectively. All the chemical and physical parameters related with reverse flow system were carefully considered and both methods were successfully applied for the determination of LOR in its dosage forms. These simple and high throughput methods could be utilized for pharmaceutical analysis of LOR.

1. Introduction

Lorazepam (7-chloro-5-(2-chlorophenyl)-3-hydroxy-2,3-dihy dro-1H-1,4-benzo-diazepin-2-one) is a benzodiazepine indicated for the treatment of anxiety disorders and preoperative sedation [1]. LOR has the chemical formula $C_{15}H_{10}Cl_2N_2O_2$ (Fig. 1).

It is applied in a wide range of indications and patient categories, because of its sedative and anticonvulsant properties. Within pediatrics, it is administered to children with status epilepticus and for intubated and mechanically ventilated patients [2]. Lorazepam is inexpensive, long acting (up to 72 h) [3] and has less risk of seizure recurrence. Lorazepam has a shelf life of 3 years and can be given intravenously (IV), intramuscularly (IM), and intranasal (IN) [4].

Determination of LOR concentration in pharmaceutical preparations was previously done using potentiometry and multi- wavelengths spectrophotometry [5], high-performance liquid chromatography (HPLC) [6–8], gas chromate-graphy- mass spectrometry (GC-MS) [9–11], micellar electrokinetic capillary chromatography [12] and voltametry [13]. Among various methods available for trace analysis, FI analysis methods provide a fast, simple and inexpensive analysis of trace amounts of different chemicals and pharmaceutical forms [14]. The literatures reported only a few FIA methods [15,16] available for the determination of LOR, one of them [15] was not specific, only one wavelength in the UV region being chosen for the spectrophotometric detection of LOR. until now the literature contains no reverse flow injection-spectrophotometric methods for the determination of LOR in pure or dosage forms. This study describes fast and simple batch and reverse FI methods as a direct methods that utilize a colorimetric reaction for the determination of LOR in pharmaceutical preparations.

2. Experimental

2.1. Apparatus

- A digital double beam spectrophotometer a type of Shimadzu UV–VIS 260 (Shimadzu, Kyoto-Japan) had been used for all spectral and absorbance measurements of FIA procedures. The absorbance measurements were carried out using 1 cm path length of quartz flow matched cells (Cecil, 50 μL internal volume), and for the absorbance measurements of the batch method, a matched 1 cm silica cells were used.
- A peristaltic pump of six channels (Ismatec, Labortechnik-Analytic, type CH-8152, Glatbrugg Zurich-Switzerland) used for pumping the solutions of reagents. A 6-ways injection valve with different loops (Rheodyne, Altex 210, Supelco -USA) was used for injected samples.

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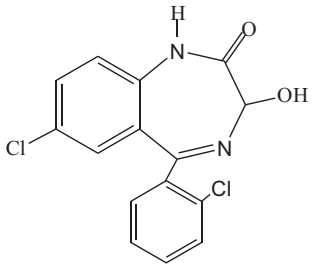

Fig. 1. Chemical structure of lorazepam.

- A flexible vinyl tubing (0.8 mm i.d.) was used for the peristaltic pump, while a tubes of Teflon material with an internal diameter of 0.5 mm was used for made different lengths of reaction coil (RC).

A two-channel manifold (Fig. 2) was employed for the RFIA spectro-photometric determination of LOR drug. The solution of drug (LOR) was transported through one of the two channels in this setup. On the other hand, the second channel was used to transport ferric chloride solution. The reagent solution of methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) was injected through the system of valve into a stream of LOR then mixed with oxidant in the reaction coil. The resultant solutions were propelled by peristaltic pump at a total flow rate of 0.6 mL/min into the detector. A green colored product was formed and absorbance was measured at 659 nm.

2.2. Materials and chemicals

An analytical grade reagents, chemicals and distilled water were used usually through the work. Pharmaceutical grade LOR and excipients were received from state company for Drug Industries and Medical Appliance, SDI, Samara-Iraq.

Pharmaceutical dosage forms containing LOR were purchased from local commercial sources. Two generic versions each contains LOR as its active constituent were used for analysis in this study (Lorativan®, 2 mg, Domina pharmaceuticals, Syria and Lorazesam®, 1 mg, SDI, Iraq).

2.2.1. LOR solution (500 μg/mL)

For batch and RFIA methods, LOR standard solution (500 μg/mL) was freshly prepared by dissolving 0.025 g of pure LOR powder in 50 mL ethanol. More dilute solutions can be prepared daily by appropriate dilution using distilled water.

2.2.2. Methylbenzothiazoline-2-one hydrazone hydrochloride (0.05 M)

The acidic solution of this reagent was prepared by dissolving 1.08 g of MBTH (Sigma Aldrich, Missouri, United States) in a 100 mL of 0.2 M HCl (Merck, Germany). More dilute solutions can be prepared daily by appropriate dilution using the same solvent (this solution is stable more than two weeks).

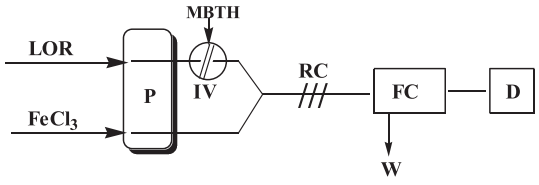

Fig. 2. Manifold employed for RFIA-Spectrophotometric determination of LOR by reaction with MBTH and $FeCl_3$, where: IV, injection valve; RC, reaction coil; P, peristaltic pump; FC, flow cell; D, detector; W, waste.

2.2.3. Ferric chloride (2% V/V)

This solution was prepared by diluted 2 mL of ferric chloride solution (60% W/V $FeCl_3$, BHD, UK) in 100 mL distilled water for batch and RFIA methods. More dilute solutions can be prepared daily by appropriate dilution using the same solvent.

2.2.4. Solutions of pharmaceutical dosage forms

Twenty Lorativan retard tablets (2 mg LOR) or forty Lorazesam tablets (1 mg LOR) were weighed and finally powdered. An amount of resultant powder equivalent to 0.0125 g of LOR was dissolved in 25 mL of ethanol and the resultant solution was shaked and filtered into a 50 mL volumetric flask, the residue was washed and diluted to volume with ethanol to obtain 250 μg/mL of LOR. More dilute solutions can be prepared daily by appropriate dilution using distilled water for batch and RFIA methods.

3. Procedures

3.1. General batch procedure

An aliquot of sample containing 20–400 μg of LOR was transferred into a series of 10 mL standard flasks. A volume of 2.0 mL of 0.02 M MBTH solution, and 1.5 mL of 2% V/V ferric chloride solution were added. The contents of the flasks were diluted to the mark with distilled water, mixed well and kept for 10 min. The absorbance was measured at 659 nm (at room temperature 25 °C) against reagent blank containing all materials except LOR. The drug concentration was computed using a standard calibration graph. For the optimization of conditions and in all subsequent experiments, a solution of 25 μg/mL was used.

3.2. General RFIA procedure

Working solutions of LOR in the range 25–400 μg/mL were prepared from stock solutions. A 150 μL portion of 0.04 M of MBTH was injected into the stream of LOR which then mixed with the oxidant solution (ferric chloride of 8% V/V), with a total flow rate of 0.6 mL/min (Fig. 2). The resulting absorbance of the green product was measured at 659 nm and a calibration graph was shown in Table 1. Optimization of conditions was carried out on 100 μg/mL of LOR.

4. Results and discussion

4.1. Reaction mechanism of the method

LOR was found to react directly with MBTH in the presence of ferric chloride as oxidant agent to produce a green colored product with absorbance measured at λ_{max} 659 nm. MBTH has been utilized for the spectrophotometric determination of several other compounds [17]. An electrophilic intermediate was formed when

Table 1
Analytical features of the procedures developed for the estimation of LOR.

Parameter	Batch procedure	RFIA procedure
Regression equation	y = 0.0166x + 0.1268	y = 0.0015x + 0.1008
Linear range (μg mL^{-1})	2–40	25–400
Correlation coefficient (R^2)	0.9965	0.9996
Limit of detection (s/n = 3) (μg mL^{-1})	0.614	2.287
Limit of quantification (μg mL^{-1})	2.047	7.624
Reproducibility %	<0.321	<0.913
Average of recovery, %	101.165	99.560
Through-put (sample per hour)	6	26

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