Simultaneous determination of sulfachloropyridazine and trimethoprim in veterinary formulations by hplc

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Abstract

In this study, a simple, sensitive, precise, rapid and reversed-phase high performance liquid chromatographic method with ultraviolet detection for simultaneous determination of Sulfachloropyridazine and Trimethoprim is developed and applied commercial veterinary formulation for chromatographic separation, Thermoscientific Hypersil-C18, reverse phase column was used. Separation was done using acetonitrile: pH 3.0 buffer solution (30:70, v/v) at 0.8 ml min⁻¹ flow rate. Analysis was done 272.0 nm at ultraviolet detector. Under optimum chromatographic conditions, retention time of Sulfachloropyridazine and Trimethoprim were determined as 4.37 and 2.60 minute. The proposed method was linear in the range 1.0-100.0 μg ml⁻¹ for Sulfachloropyridazine and 0.5-40.0 μg ml⁻¹ for Trimethoprim. The Limit of detection of Sulfachloropyridazine and Trimethoprim are 0.05 μg ml⁻¹, 0.18 μg ml⁻¹, respectively. The method which is rapid, simple and does not require any separation step, has been successfully applied to assay of commercial dosage forms containing Sulfachloropyridazine and Trimethoprim.

Keywords:
- Sulfachloropyridazine
- Trimethoprim
- HPLC

1. Introduction

Sulfonamides that called sulfa drugs block folic acid synthesis in bacteria and coccidian by emulating para amino butyric acid. Sulfa drugs were the first chemical substances systematically used to treat and prevent bacterial infections in humans and animals [1]. Sulfachloropyridazine (SCP) is a member of Sulfonamide, effective against many microorganisms. SCP is well tolerated by animals and it is only used for animals. Combination of Sulfa drugs with Trimethoprim (TMP) have a wide spectrum on bacteria and coccidiosis. The using combination of sulfa drugs and TMP have bacteriocidal effect on microorganism [2, 3]. Antibiotic combinations are frequently used for indications such soft tissue infections, shigellosis, diarrhea, bronchitis for human and animals [4, 5].

Several techniques have been presented for determination of SCP and TMP separately or simultaneous with other drugs in veterinary, pharmaceutical or biological samples such as HPLC [6-10], spectrophotometer [11-14], LC/MS/MS [15-18], capillary electrophoresis [19, 20].

Ni et al. (2006) applied spectrophotometric determination method for sulfonamides by multivariate calibration approaches. Their approaches has the aid of chemometrics methods comparing the classical least squares, principal component regression and partial least squares models [21]. This methods requires the application of chemometrics.

Pokaola et al. (2018) presented UV spectrophotometric method for the simultaneous analysis of Sulfadiazine [SDA] and Trimethoprim [TMP] in pharmaceutical formulations. The advantages of Pokaolas method according to analytical purposes that rapid determination, cost-effectiveness and easy preparation. The developed method has not been requirement to chemometrics approaches [22]. Abd-Alrassol presented (2017) new spectrophotometric determination method for sulfamethoxazole and sulfamerazine in bulk and pharmaceutical dosage forms by a chemical derivatization method involving proton transfer [23]. The spectrophotometric methos have important disadvantages that, matrix effect and analysis time. Liquid chromatographic methods applies simultaneous separation and minimum matrix effects for drug analysis in different sample matrix. Tahan et al. (2015) developed simultaneous determination method of Sulfamethoxazole (SMZ) and Trimethoprim content in veterinary drugs by HPLC. The diode array detector was used determination for TMP and SMZ [24]. Goulas et al. (2014) applied HPLC method for the simultaneous determination of sulfadimidine, sulfadiazine, sulfamethoxazole and trimethoprim antibiotics in medicated feeds has been developed.

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analysis method to commercial feed premixes after the ultrasound-assisted extraction procedure [25].

The aim of this study was to investigate simultaneous determination of SCP and TMP combination in veterinary formulations by HPLC. This study described the proposed chromatographic method for simultaneous analysis SCP and TMP in veterinary formulations. The developed method must have properties such as simple, fast and sensitive for determination and quality control in drug formulation. The time-consuming and pre-treatment procedures weren’t prefer to propose a widely applicable routine analysis method.

2. Materials and Methods

2.1. Instruments and reagents

The chromatographic system is consisted of the commercial components: an Agilent isocratic LC pump, an automatic sample injector system (Agilent 1260 Infinity) and photodiode array detector (Agilent Model G1315D). For chromatographic separations was used Thermoscientific Hypersil C18 column (150 mm x 4.6 mm i.d.). SCP and TMP were obtained by Novartis (Ankara, Turkey). HPLC purity methanol, acetonitrile (Merck, Germany) and orthophosphoric acid (Sigma-Aldrich, Germany) were used. Deionized water was provided by using Millipore Elix® 5 UV resistant 14 MΩcm$^{-1}$.

2.2. Chromatographic analysis

Chromatographic separation was practiced at room temperature. The SCP and TMP were separated using by mobile phase mix of ACN: pH 3.0 buffer solution (30% ACN: 70% pH 3.0 buffer solution). The mobile phase was prepared daily and filtered, sonicated before using. The flow rate for separation was setted as 0.8 ml min$^{-1}$ and the chromatogram was monitored at 272 nm. Injection volume of sample and standard was done as 25 µl.

2.3. Preparation of standards and sample solutions

Standard SCM and TMP solutions were prepared in methanol at concentration of 1 mg ml$^{-1}$ for calibration curve and sample solutions. The standard solutions were provided by diluted mobile phase at different concentrations range of 1.0-100.0 µg ml$^{-1}$ for SCP and 0.5-40.0 µg ml$^{-1}$ for TMP.

2.4. Validation of the proposed chromatographic method

The developed method was validated according to The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) method validation guidelines. The linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness were investigated for the validation of developed chromatographic method. Precision was examined interday (n=6), and intradays (n=5). The accuracy and precision of the proposed method were presented by recovery studies and %RSD, respectively.

2.5. Application of veterinary formulation

1 ml of the oral solution was obtained and diluted to a hundred ml with HPLC grade methanol. Sample solutions were prepared from diluted oral veterinary formulation. The optimum chromatographic conditions for the HPLC method were applied to determination of SCP and TMP in veterinary formulation. Recovery studies were performed by the standard addition of known amounts of SCP and TMP to a known concentrations of the commercial formulation.

3. Results and Discussion

At large quantities mobile phase solvents were prepared and used to provide an optimum chromatographic separation such as methanol, acetonitrile, buffer solutions and deionize water. Mobile phase solvents were examined various mixture and percentage. The mixture of ACN: pH 3.0 buffer solution (30:70, v/v) gave better separation and peak symmetry for SCP and TMP. The optimum injection volume was decided to 25 µl according to peak symmetry and shape of peak. The retention time for TMP and SCP were found as 2.60, 4.37 min (Fig.1) at optimum chromatographic conditions.

Fig. 1. Chromatogram of simultaneous analysis of SCP and TMP.
3.1. The Results of System suitability tests of SCP and TMP for the proposed method

The system suitability tests are an important part of a liquid chromatographic method. Results of system suitability tests were explained in Table 1. Freshly prepared standard solution of SCP and TMP were used for system suitability tests. The most important system suitability parameter in liquid chromatography is to assure the optimum resolution in the minimum separation time. A resolution value of 1-1.5 or greater between two peaks will provide that the sample components are separated. Resolution of SCP and TMP was found 14.4 in proposed method thus optimum resolution was carried out for sample component.

When capacity factors are very high or very low, the quality of the chromatographic separation is decreased. The capacity factors were obtained as 1.42 for SCP and 0.457 for TMP at optimum separation conditions. All chromatographic peaks would be symmetrical and the peak symmetry ratio is to obtain ≤ 1.5 for acceptable peak shape. The peak symmetry ratio of SCP and TMP are acceptable for HPLC method. The number of theoretical plates (N) is frequently used to correlate the efficiency of a column for a developed method. The number of theoretical plates must to be ≥ 2000 for each component according to ICH. The developed method carried out sufficient separation at column by value of N of SCP and TMP. The % RSD of retention times SCP and TMP have been 0.21% and 0.14 % and results show a well precision at optimum separation conditions.

| Table 2. Analytical performance of the developed method |
|-----------------|-----|-----|
| Validation parameters | SCP | TMP |
| Linear dynamic range (mg ml⁻¹) | 1.0-100.0 | 0.5-40.0 |
| Slope | 113.72 | 30.03 |
| Intercept | 17.18 | 24.51 |
| r² | 0.9998 | 0.9969 |
| RSD of slope | 1.52 | 1.59 |
| RSD of intercept | 6.20 | 4.43 |
| LOD (mg ml⁻¹) | 0.05 | 0.18 |
| LOQ (mg ml⁻¹) | 0.16 | 0.60 |

3.3. The results of application of real sample

The recoveries study were carried out different concentration levels sample components for the measurement of accuracy of the developed HPLC method. The mean recovery values of developed HPLC method were shown in Table 3. The recovery values were obtained the range of 99-105% for SCP and TMP. The accuracy of developed method is acceptable according to analytical parameter.

| Table 3. Recovery values of SCP and TMP in veterinary formulation |
|-----------------|-----|-----|
| Added analyte concentration µg ml⁻¹ | Results µg ml⁻¹ | Mean Recovery |
| SCP | | |
| 0.0 | 27.7 ± 0.5 | - |
| 22.3 | 49.9 ± 0.4 | 99.6 ± 1.8 |
| 27.8 | 55.4 ± 0.7 | 99.6 ± 2.6 |
| 33.4 | 61.6 ± 0.1 | 101.5 ± 0.2 |
| TMP | | |
| 0.0 | 5.9 ± 0.1 | - |
| 4.8 | 10.8 ± 0.1 | 102.1 ± 2.4 |
| 6.0 | 12.2 ± 0.1 | 105.0 ± 1.1 |
| 7.2 | 13.1 ± 0.1 | 100.0 ± 0.7 |

The results of analysis of veterinary formulation were presented in Table 4. The values of result for veterinary formulation are acceptable according to labelled value of drug sample. Therefore the developed method could be use quality control analysis and simultaneous determination of SCP and TMP.

3.2. Analytical performance of the proposed chromatographic method

By application this method linear dynamic range were observed between the peak area and the concentration of 1.0-100.0 µg ml⁻¹ for SCP and 0.5-40.0 ml⁻¹ for TMP. Table 2 represents calibration characteristic and analytical performance parameters for the proposed method. The LOD and LOQ of the developed method were presented in Table 2, then were calculated 3.0 µ/s and 10 µ/s according to ICH [26]. The precision of the developed method was calculated for interday precision and intraday precision. The interday precision was determined ≤ %0.5 for SCP (n=6) and ≤ %2.3 for TMP, intraday precision was determined ≤ %1.0 and ≤ % 2.5 respectively.

| Table 1. System suitability parameters of SCP and TMP (n=6) |
|-----------------|-----|-----|
| System suitability parameter | SCP | TMP |
| Retention time (min) | 4.37 | 2.60 |
| % RSD of Retention time | 0.23 | 1.54 |
| Resolution | 14.4 | 14.4 |
| Peak symmetry ratio | 0.551 | 0.516 |
| Capacity Factor | 1.42 | 0.457 |
| The number of theoretical plate | 13191 | 12954 |
| % RSD of precision injection (n=10) | 0.09 | 0.39 |
Table 4. Result of the determination of SCP and TMP in veterinary dosage form.

| Active Compound | Labelled Claim (mg) | Mean of amount found (mg)* |
|-----------------|---------------------|---------------------------|
| SCP             | 100.0               | 92.2±1.5                  |
| TMP             | 20.0                | 19.5±0.3                  |

*Mean value of six determination

The proposed chromatographic method was compared to other analytical methods in literature at Table 5. The developed HPLC method is first for simultaneous determination of SCP and TMP. The proposed chromatographic method is sufficient accuracy and precision to be analyzed to veterinary formulation.

Table 5. Comparison of the proposed method with other analytical techniques

| Drug molecules | Sample | Instrument | LOD (µg/ml) | % RSD | Ref. |
|----------------|--------|------------|-------------|-------|------|
| Sulfadiazine, sulfadimidine, sulfamethoxazole, sulfanilamide and trimethoprim. | Pharmaceutical and veterinary formulations | UV/Vis spectrophotometer by multivariate calibration approaches | 240.0 - 810.0 | 3.1 - 6.34 | [21] |
| Sulfadiazine and Trimethoprim | Pharmaceutical formulations | UV/Vis spectrophotometer | 0.82 - 0.25 | 2.3 - 2.1 | [22] |
| Sulfamethoxazole and sulfamerazine | Bulk and pharmaceutical dosage | UV-Visible spectrophotometer | 220.0 - 670.0 | 1.6 - 1.5 | [23] |
| sulfamethoxazole and trimethoprim | Veterinary medicines | HPLC-DAD | ---- | 0.99 - 2.7 | [24] |
| sulphadimidine, sulfadiazone, sulphamethoxazole and trimethoprim | feed premixes | HPLC-UV | ---- | 1.3 - 4.16 | [25] |
| Sulfachloropyridazine and trimethoprim | Veterinary formulation | HPLC-UV | 0.05 - 0.18 | 1.0 - 2.5 | This work |

4. Conclusions

The developed HPLC method is simple, fast, reliable and validated for simultaneous determination of SCP and TMP at first time. This method has a well resolution between SCP and TMP moreover the analysis time is very short. The proposed chromatographic methods accuracy, precision and the limit of detection values are particularly satisfactory and comparable with more other analytic protocols. Thus developed method could be suggested for quality control analysis of and determination of veterinary formulation which are containing SCP and TMP.

Conflicts of interest

The authors state that did not have conflict of interests.

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