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Short communication

Quantitative estimation of sultamicilln *p*-toluenesulfonate in pharmaceutical preparations by reverse-phase high performance liquid chromatography

A.P. Argekar*, S.S. Kunjir

Analytical Laboratory, Department of Chemistry, The Institute of Science, 15 Madam Cama Road, Mumbai 00 032, India

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1. Introduction

Sultamicillin *p*-toluenesulfonate is a pro-drug ampicillin and sulbactam. Its chemical structrue is as shown in Fig. 1. During absorption from the gastro intestinal tract it is hydrolysed releasing equimolar quantities of ampicillin and sulbactam which are a wide spectrum antibacterial and a beta lactamase inhibitor respectively [1]. A literature survey reveals that there is no reported method for the estimation of sultamicillin *p*-toluenesulfonate in the dosage forms. Therefore, an attempt to develop a new analytical method for this drug has been undertaken. The results obtained are reported in this paper.

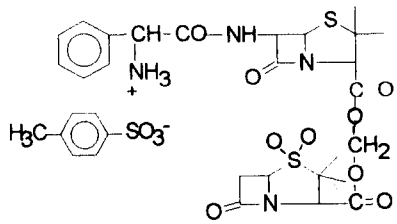

Fig. 1. Chemical structure of the sultamicillin tosylate.

2. Experimental

2.1. Instrumentation

A High Pressure Liquid Chromatograph TOSHO CCPE pump equipped with a Rheodyne 20 μl fixed loop and an SIC 12 Chromatorecorder was used. The injection volume was 20 μl.

2.2. Solvents and chemicals

A reference standard of sultamicillin *p*-toluenesulfonate was procured from Merind Ltd. (Bombay, India). Tablet formulations were procured

* Corresponding author.

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from the local market. Analytical-grade phosphoric acid was used. Acetonitrile and water were of HPLC grade, supplied by S.D. Fine chemicals Ltd. (Thane, India).

2.3. Mobile phase

0.02 M sodium dihydrogen phosphate and acetonitrile were mixed in a ratio of 60:20 (v/v) and the pH was adjusted to 3.0 with dilute phosphoric acid.

2.4. Stationary phase

A C18 Shodex column (5 μm, 250 mm × 3.9 mm i.d.) was used.

2.5. Standard stock solution

50 mg of sultamicillin *p*-toluenesulfonate (accurately weighed) was taken in a 50 ml volumetric flask, dissolved in 30 ml of acetonitrile, sonicated for about 15 min and then diluted to volume with acetonitrile.

2.6. Working standard solution

2 ml of the stock solution was diluted to 50 ml in a 50 ml standard volumetric flask with acetonitrile.

2.7. Sample solution

20 tablets were weighed and finely powdered and an accurately weighed powder sample equivalent to one tablet was placed in a 100 ml standard volumetric the flask. 50 ml acetonitrile was added and the flash was kept in an ultrasonic bath for 10 min. The sample was then diluted to the mark with acetonitrile and mixed. After filtering through a Whatman No. 42 paper a 1 ml aliquot of the filtrate solution was taken in a 100 ml volumetric flask diluted to the mark with acetonitrile and used for the analysis.

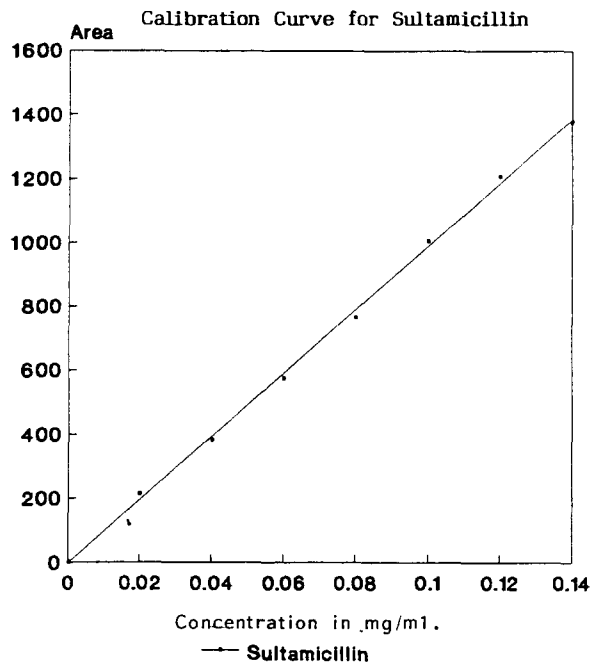

Fig. 2. Calibration curve for sultamicillin.

2.8. Calibration

Aliquots of the standard stock solution of sultamicillin *p*-toluenesulfonate were taken in different 10 ml standard volumetric flasks and diluted to the mark with the mobile phase such that the final concentrations of sultamicillin *p*-toluenesulfonate were in the range of 20–180 μg. Evaluation of the drug was performed with a UV detector at 215 nm. Peak areas were recorded for all the peaks. The calibration curve is shown as Fig. 2.

2.9. Assay

Each of the standard and sample solutions were injected into the chromatograph and peak areas were recorded as described in the previous section. From the peak area of sultamicillin the amounts were computed by an external standard method.

3. Results and discussion

3.1. System suitability

To ascertain the effectiveness of the proposed method, system suitability tests were carried out on freshly prepared standard solutions of sultamicillin *p*-toluenesulfonate. The results obtained are shown in Table 1 which indicates that the chromatographic systems are adequate for the analysis.

3.2. Chromatography

The mobile phase resolved *p*-toluenesulfonate and sultamicillin very efficiently, as shown in Fig. 3. The retention times were 3.7 and 7.8 min respectively.

3.3. Linearity and limits of detection and determination

The plots of peak area versus the respective concentration of sultamicillin were found to be linear in the range 20–180 μg ml^{-1}, with a coefficient of correlation of $r = 0.99$. The intercept values were not significantly different from zero. They were represented by the linear equation

$$Y(\text{sultamicillin}) = 9902.3X - 2.91666 \quad (r = 0.999)$$

The limit of detection (LOD) and the limit of quantification (LOQ) of sultamicillin were calculated from the peak area using the following equations:

$$\text{LOD} = 3 \times N/B \qquad \text{LOQ} = 10 \times N/B$$

Table 1
Results of system suitability for sultanicillin

Sr. No.	Parameter	
1	Tailing factor	1.23
2	LOQ (μg ml^{-1})	40
3	LOD (μg ml^{-1})	10
4	HETP	8000
5	Calibration range (μg ml^{-1})	20–180
6	Relative retention time	0.47
7	Resolution	16

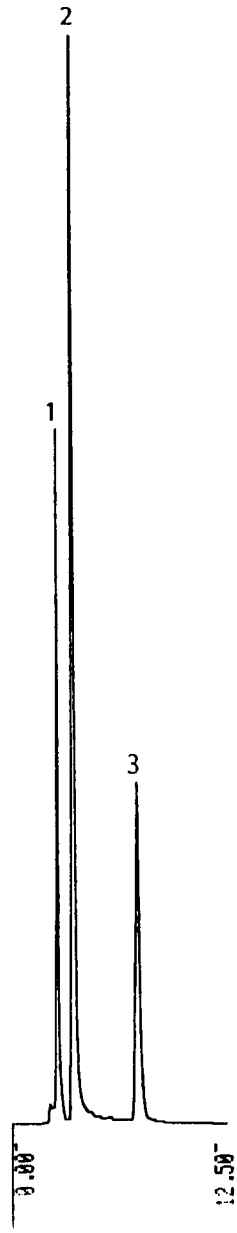

Fig. 3. Typical chromatogram showing separation of (1) solvent front, (2) *p*-toluenesulfonate, (3) sultamicillin.

where N, the noise estimate, is the standard deviation of the peak areas (five injections) of the drugs and B is the slope of the corresponding calibration curve. LOQ and LOD for sultamicillin were found to be 40 μg and 10 μg respectively.

Table 2
Results of HPLC assay of tablet formulations

Parameter	Dosage formulation	
	Tablet brand I	Tablet brand II
Component	Sultamicillin	Sultamicillin
Labelled claim (mg per tablet)	375.0	375.0
Amount found (mg per tablet)	376.02	375.20
%RSD, $n^a = 5$	1.34	1.02

[a] n = average of five experiments

Table 3
Precision of the assay method

Dosage formulation	Component	Label claim (mg per tablet)	Amount found (mg per tablet)	%RSD, $n^a = 3$
Within-day study				
Tablet	Sultamicillin	375.0	374.80	0.98
Day-to-day study				
Tablet	Sutlamicillin	375.0	374.65	0.99

[a] n = average of three experiments

3.4. Assay

The sultamicillin contents per tablet for two commercial bands found by the proposed method were in good agreement with the labelled contents, as shown in Table 2. The low RSD values indicate that the method is precise.

3.5. Accuracy and precision

The precision of the method was evaluated by repeated assays of the commercial formulations. Within-day precision was determined by performing five consecutive assays within a period of 8 h and the day-to-day repeatability of the method was determined by analysing the sample (single operator) on consecutive days (Table 3).

The accuracy of the proposed method was evaluated by recovery experiments, using the standard addition technique by adding four different levels of standards to the preanalysed sample. Each level was repeated thrice.

A plot was made of the amount of the drug found (mg) by the proposed method (y-axis) against the amount of the standard drug added (x-axis). The intercept on the y axis indicates the amount of drug present (mg) per tablet. From the amount of the drug found, the percent recovery was calculated.

The recoveries of sultamicillin obtained were 99.77% (as shown in Table 4), which shows that there is no interference from the excipients present in the tablets. A placebo tablet solution (absence of active substance) was injected, but did not show any absorbance.

4. Conclusion

The proposed RP-HPLC method is simple, precise, rapid and selective for the determination of sultamicillin tosylate and can be employed for its assay in dosage forms.

Table 4
Results of the recovery analysis

Dosage	Component	Label claim (mg per tablet)	Amount of drug added (mg)	Amount recovered (mg per tablet)	% Recovery	% Mean recovery
Tablet	Sultamicillin	375.0	0.00	375.10	99.49	99.77
			20.00	395.53	100.10	
			40.00	412.10	99.27	
			60.00	436.08	100.22	

Acknowledgements

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References

[1] Martindale: The Extra Pharmacopeia, 30th edn., The Pharmaceutical Press, London, 1993, p. 211.