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A validated HPLC method for the determination of thiazinamium methylsulphate in pharmaceutical preparations

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Abstract

The phenothiazine derivative thiazinamium methylsulphate is a drug with antihistaminic and anticholinergic properties, often used in some types of obstructive lung diseases. Because there is a lack of chromatographic data available for its determination, the objective of the present investigation was to develop a sensitive and rapid HPLC method for the quantitative estimation of thiazinamium methylsulphate in a pharmaceutical dosage form, applicable to routine analysis. The drug was chromatographed on a C18-reversed phase system applying a Licrocart column (LiChrospher 100 RP 18, 125 × 4 mm) with a mobile phase consisting of acetonitrile–water (3:7, v/v), employing as ion-pairing agent octanesulphonic acid sodium salt (20 mM) together with *N*,*N*-dimethyloctylamine (20 mM), adjusted to pH 3. Detection occurred at 254 nm. Propylparaben was used as an internal standard. The method was applied to solutions for intramuscular injection containing thiazinamium methylsulphate (65 mg/2 ml). Since little sample preparation is required, most analyses can be carried out within 15 min. The optimized method was validated and provided acceptable results with respect to linearity ($r = 0.9999$), precision and accuracy in the concentration range of 26–78 μg/ml. The proposed method is presently employed to investigate the stability of thiazinamium methylsulphate in solutions for intramuscular injection in the presence of anti-oxidizing agents. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: High perfomance liquid chromatography; Thiazinamium methylsulphate; UV-detection; Pharmaceutical analysis; Promethazine . HCl; Promazine . HCl; Validation of HPLC method

1. Introduction

Thiazinamium methylsulphate (TMS) (10-(2-trimethylammonium-propyl)-phenothiazine-methylsulphate) (Fig. 1) is a phenothiazine drug with a quaternary ammonium group in the side chain.

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TMS has antihistaminic and anticholinergic properties [1], the latter being responsible for the bronchodilatation when administered to patients suffering of chronic respiratory diseases. Monitoring serum concentrations of TMS after intramuscular, rectal and oral administration shows that solutions for intramuscular injection are the most effective dosage forms. An important fraction of thiazinamium is metabolized in the liver after oral administration. On the other hand, intramuscular administration can cause painful infiltrates [2].

Few methods for the determination of TMS have been published. These include gas chromatography [3] and thin layer chromatography [4]. The TLC method can be used for the quantitative determination of the cation (thiazinamium$^+$) compound, but for routine analysis it is preferable to employ a more sensitive method. Though the GC method of Jonkman et al. [3] is capable of measuring concentrations in the nanogram range, two structure analogues of TMS, prometazine and chlorpromazine are found to interfer with TMS using their procedure. Liquid chromatographic methods are also available in literature. Recently, our group achieved a chiral separation of phenothiazine compounds on a chiralcel OJR column [5]. Greving et al. [6] investigated the separation of quaternary ammonium compounds based on ion-pair adsorption HPLC.

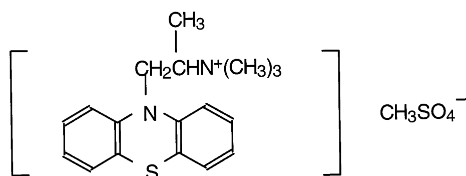

Fig. 1. Thiazinamium methylsulphate.

Formulation:

Thiazinamium methylsulfate		**65**	**mg**
EDTA.2Na		**1**	**mg**
Ascorbic acid		**2**	**mg**
Sodium chloride		**14**	**mg**
Water	**ad**	**2**	**ml**

Fig. 2. The composition of the injectable solution.

Both LC methods were carried out on a normal phase column.

In this paper, we report a sensitive reversed phase HPLC method for the quantitative determination of TMS in solutions for intramuscular injection. The method is applicable as well for routine analysis. It is based on the ion-pairing properties of octanesulphonic acid sodium salt (SOS) and *N*,*N*-dimethyloctylamine (DMOA) on a RP 18 column and UV-detection at 254 nm. The presented method complies with the validation requirements in the pharmaceutical industry.

2. Experimental section

2.1. Reagents and chemicals

HPLC grade acetonitrile (ACN) was obtained from Pancreac-Química (Spain). Octanesulphonic acid sodium salt (Aldrich-Chemical, England) and *N*,*N*-dimethylamine (DMOA) (Janssen Chimica, Belgium) were used as ion-pairing agents. Thiazinamium methylsulphate was obtained from Alfa Pharma (Belgium) and the internal standard propylparaben was purchased from Federa (Belgium). Deionised water was used throughout. The other chemicals used were all of analytical grade.

2.2. Instrumentation

Chromatography was performed on a Varian 9010 SDS pump (Varian Associates Inc., USA) using a Rheodyne 7125 injector with a 20 μl loop. Detection was performed with a Hewlett Packard series 1050 diode array detector (Hewlett Packard, Germany). Integrations of the chromatograms were made with the Hewlett Packard software package. For the present study the following column was applied: 125 × 4 mm Licrospher RP-18, 5 μm particle size (Merck).

2.3. Mobile phase

Twenty millimolar SOS and 20 mM *N*,*N*-DMOA were dissolved in an acetonitrile:water mixture (7:3, v/v). The pH of the mobile phase

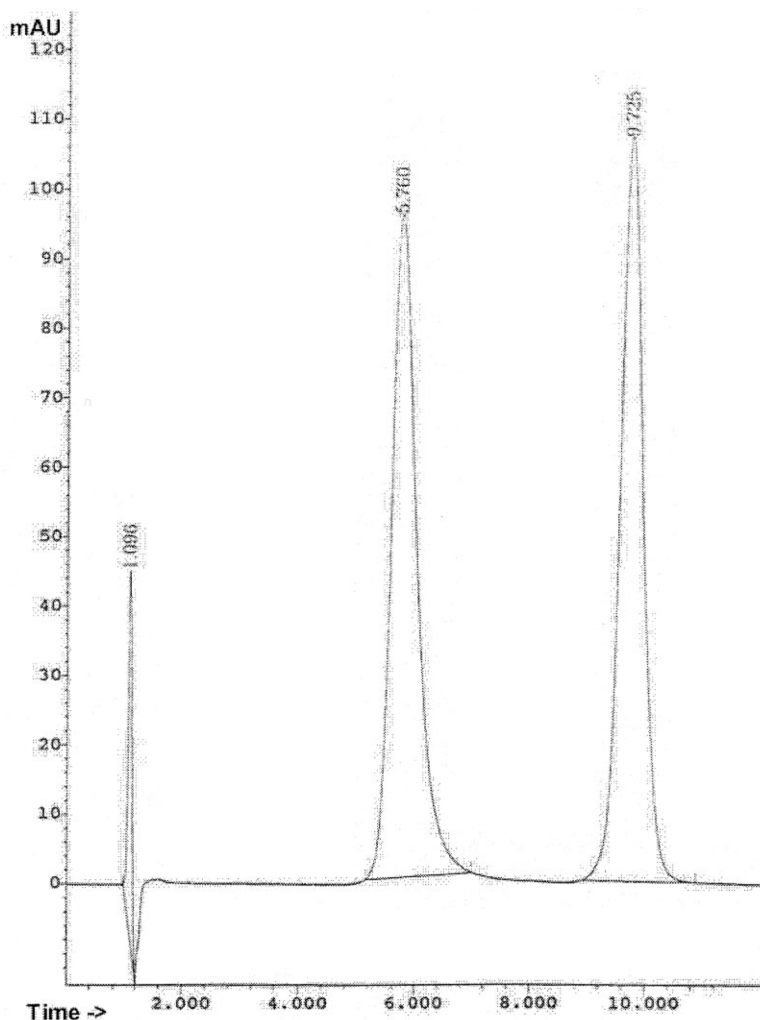

Fig. 3. A typical chromatogram obtained after injection of the sample solution (retention times: TMS, 5.8 min; propylparaben, 9.7 min).

was adjusted to 3.0 with diluted phosphoric acid (20%, v/v).

2.4. Chromatographic conditions

The mobile phase was pumped at a flow rate of 0.8 ml min^{-1}. Chromatography was carried out at 35°C. The column eluate was monitored at 254 nm, a suitable wavelength obtained from the TMS UV-spectrum.

2.5. Standard solution preparation

Stock standard solutions of TMS (1.3 mg ml^{-1}) were prepared in methanol–water mixture (6:4, v/v), in three-fold. Working standard solutions were achieved by diluting each stock standard solution with the methanol–water mixture (6:4, v/v) resulting in concentrations of 0.13–0.39 mg ml^{-1}. For injection, 200 μl working standard solution was mixed with 800 μl mobile phase.

stationary phase
LiChrocart 125 mm x 4 mm (guard kolom 4-4)
packed with LiChrospher 100 RP 18, 5 μm I.D.
Merck Belgolabo

mobile phase

acetonitrile	300 ml
water	700 ml
SOS	20 mM
DMOA	20 mM

brought to pH 3.0 with diluted phosporic acid

flow rate:
0.8 ml min^{-1}

injection volume:
20 μl
column temperature
35 °C

UV-detector
HP-1050 diode-array detector
flow-cell volume 5 ul
lengte 10 mm

detection wavelength
254 nm

Scheme 1. Operating conditions.

A calibration curve of peak areas as a function of TMS concentration was established in the range of 26–78 μg ml^{-1}, in the presence of propylparaben (240 μg ml^{-1}) as internal standard.

2.6. Sample solution preparation

Dilutions of the injectable solutions (label claim: 32.5 mg ml^{-1}, composition: Fig. 2) were prepared in methanol–water mixtures (6:4, v/v) down to a concentration of ± 260 μg ml^{-1}. For injection, 200 μl of the latter solution was mixed with 800 μl mobile phase in order to obtain a final concentration of 52 μg ml^{-1}, in the presence of propylparaben (240 μg ml^{-1}) as internal standard.

2.7. Stability study

Three batches of intramuscular solutions (label claim: 32.5 mg ml^{-1}) were studied at two different temperatures (room temperature and 40°C) for 15 months. Each sample was assayed to determine the concentration of the active ingredient present immediately before storing. Additional assays for each temperature were performed at specific time intervals (0, 3, 9, 12 and 15 months).

2.8. Calculations

Calibration graphs were constructed by plotting the peak area ratios TMS/internal standard of the standard solutions versus concentration of the standard solutions and analysed by least-squared regression.

3. Results and discussion

3.1. Preliminary experiments

As the drug studied has high water solubility and ionizable properties, the ion-pair reversed phase mode was the first choice for the present

Table 1
Data of the recovery experiments

Spiking level % theoretical content	Assay no.	Recovery (%)	Mean	RSD
50	1	97.6		
	2	101.5	100.1	2.2
	3	101.2		
100	1	99.2		
	2	100.2	100.6	1.6
	3	102.3		
150	1	100.0		
	2	98.5	99.8	1.2
	3	100.9		

Table 2
Obtained retention times and resolutions for the investigated compounds applying three different columns[a]

	LiCrocart 125×4 mm, Licrospher 100 RP 18		BIO-SIL 250×4.6 mm, C18-5S		BIO-SIL 250×3.2 mm, C18-5S	
TMS (a)	t_1	6.2	t_1	9.6	t_1	7.2
Propylparaben (b)	t_2	10.4	t_2	14.3	t_2	10.9
Resolution a-b	6.0		8.7		6.7	
TMS (a′)	t_1	6.1	t_1	11.2	t_1	7.3
Promethazine hydrochloride (b′)	t_3	9.7	t_3	18.4	t_3	11.8
Resolution a′-b′	3.4		7.9		5.9	
Resolution a′-c′	8.5		10.4		8.1	
Flow	0.8 ml/min		2.1 ml/min		1 ml/min	

[a] t_1, t_2 and t_3: Retention times in minutes.

Table 3
Influence of pH of the mobile phase on the resolution of the investigated compounds[a]

	pH 2	pH 3	pH 4	pH 5
Resolution TMS-propylparaben	5.8	5.3	5.0	4.8
Resolution TMS-promethazine . HCl	4.0	4.3	4.3	4.5
Resolution promethazine . HCl-promazine . HCl	1.6	1.6	1.5	1.4

[a] Conditions: mobile phase: 30% acetonitrile (v/v), 70% water (v/v), 20 mM DMOA and 20 mM SOS; column temperature: 35°C.

investigation. Based on previous work on ion-pair liquid chromatography of other quaternary ammonium drugs [7–10], two counter-ions of opposite charge were used resulting in efficient, selective and well-resolved separations. Counter-ions are added primarily to reduce interactions of the analytes with accessible residual silanol groups, which otherwise would result in tailing, poorly resolved and long retained peaks. Most of the time, however, the pairing ion is just a single ion. The commonly observed assymmetry in tailing of solute peaks in reversed phase ion-pair chromatography is generally attributed to competing secondary equilibria, such as dissociation of the pairing ion. Using a combination of two ion-pairing agents, this problem can be solved.

The starting mobile phase consisted thus of the following components: a methanol: water mixture (6:4, v/v) in which 10 mM DMOA was dissolved together with 20 mM SOS, all brought to pH 3 with diluted phosphoric acid. In order to obtain a very selective separation method, a mixture of all phenothiazine derivatives (TMS, prometazine . HCl and promazine . HCl) was injected. TMS was the first eluting component at 7.7 min, giving a retention factor of 5.3. This was acceptable as the main goal of this study included the development of a selective method to determine only TMS in pharmaceutical preparations.

In the next step methanol was replaced by the same amount of acetonitrile to yield better selectivity. By systematically lowering the content of acetonitrile in the mobile phase, a more suitable separation of the three peaks was reached at 30%, v/v acetonitrile. Lower amounts are not recommendable because of too long retention times.

To shorten the retention times, the influence of DMOA was studied. As expected, the higher the DMOA concentrations in the mobile phase, the shorter the retention times.

The final composition of the mobile phase consisted of 30% acetonitrile (v/v), 70% water (v/v), 20 mM DMOA and 20 mM SOS, brought at pH 3 with diluted phosphoric acid. The retention time

of TMS was of 5.8 min. A considerable equilibration time has to be considered due to the two counter-ion concentrations.

Finally it was decided to introduce an internal standard for the quantification of TMS in the injectable solution, in view of switching to microbore HPLC in the future. When looking for the right internal standard for this method, the structure analogue promethazine . HCl was found to be a useful candidate: having similar chemical structure, detectable applying the same method, having more or less the same UV-spectrum and moreover, having not too different retention times. The compound was considered suitable. Its

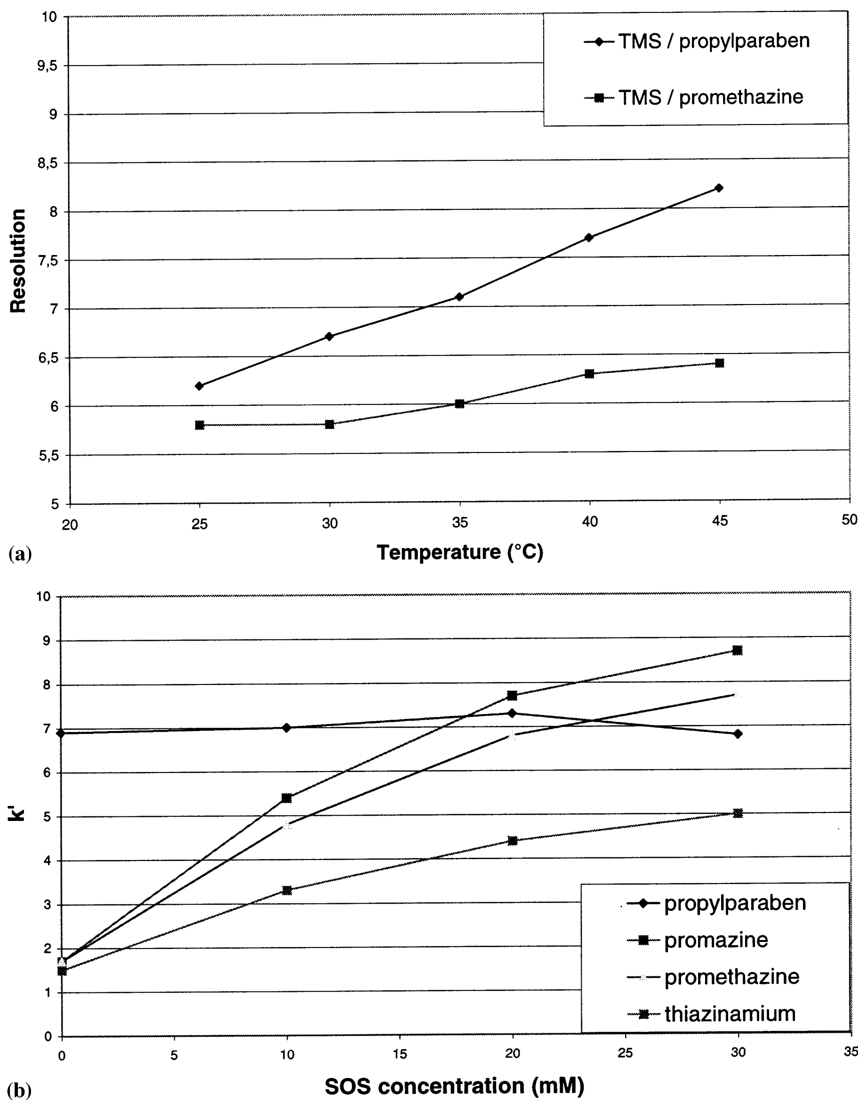

Fig. 4. Influence of column temperature on the resolution of the investigated compounds (conditions: mobile phase: 30% acetonitrile (v/v), 70% water (v/v), 20 mM DMOA and 20 mM SOS, brought at pH 3 with diluted phosphoric acid). (b) Influence of SOS concentration in the mobile phase on the capacity factors of the investigated compounds (conditions: mobile phase: 30% acetonitrile (v/v), 70% water (v/v), 20 mM DMOA, brought at pH 3 with diluted phosphoric acid; column temperature: 35°C). (c) Influence of acetonitrile content in the mobile phase on the resolution of the investigated compounds (conditions: mobile phase: 20 mM DMOA and 20 mM SOS, brought at pH 3 with diluted phosphoric acid; column temperature: 35°C).

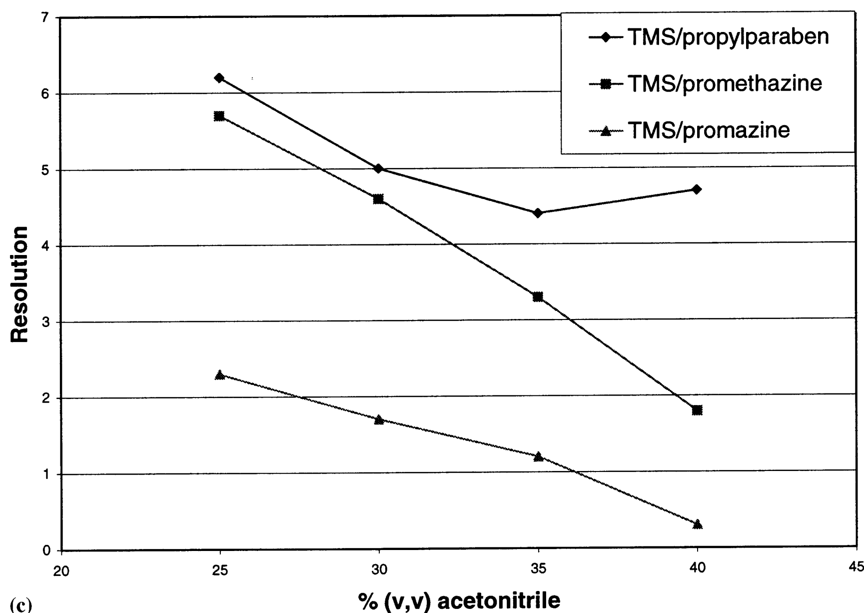

Fig. 4. (*Continued*)

Table 4
Residual amount of TMS in ampoules under two storage temperatures for 15 months

Batch		Residual amount of TMS(%)				
		Right after production	3 months	9 months	12 months	15 months
98C11	RT	100.5	99.1	100.0	101.5	100.2
	40°C		101.4	99.7	98.8	101.2
98C13	RT	100.6	98.3	100.6	101.2	100.9
	40°C		99.2	100.2	100.5	101.0
98D02	RT	99.8	99.5	100.5	98.2	100.5
	40°C		98.3	98.9	99.2	100.8

use as an internal standard, however, was ommited because of the unstability properties of this drug in solutions, as break-down products could be similar as for TMS. The preservatives methyl-, ethyl-, and propylparaben were tested using the final mobile phase composition. Almost each paraben compound could be utilised since none elutes close to TMS. However, ethylparaben was omitted because of coelution with the artefact peaks appearing in a degraded TMS solution.

3.2. Method validation for the determination of TMS in the studied dosage form

A typical chromatogram obtained from a sample preparation is illustrated in Fig. 3.

To demonstrate that this method has a performance that can guarantee reliable results, the method was validated with respect to linearity, precision, accuracy, specificity, sample solution stability and robustness under the selected operating conditions (Scheme 1).

3.2.1. Specificity

The specificity of the method was investigated by observing potential interferences between TMS and the internal standard, propylparaben. No interfering peaks were present. This HPLC method allows the direct determination of TMS in the presence of the excipients, as no interferences with the latter can be observed. Because no degradation products of TMS are known, TMS standard solutions (25 μg ml^{-1}) were stressed by thermal, acidic, basic, oxidative and UV light environments for a fixed period of time. No interfering peaks at the retention time of TMS were observed in any of the stressed samples.

3.2.2. Precision

Repeatability of successive injections was determined by injecting the same standard solution for eight times giving an RSD of 0.62% (peak area ratio) and by injecting eight times the same sample solution giving an RSD of 0.90% (peak area ratio).

Analysis repeatability was assessed in combination with the accuracy study (see Section 3.2.3). Recovery data in triplicate at each level (50, 100 and 150%) are given in Table 1. The RSD of the replicates shows that the studied method provides acceptable precision.

3.2.3. Accuracy

The accuracy of the method was shown by analysing placebo solutions spiked with known amounts of TMS corresponding with 50, 100 and 150% of the label claim. These experiments were assessed on 3 different days by the same analyst. The mean of the replicates indicates how accurate the method is. The recovery results for TMS in the specific formulation are shown in Table 1.

3.2.4. Linearity

A five point-calibration curve shows the linearity of the response, relative peak areas TMS/propylparaben versus TMS concentration. This was assessed with five standard solutions corresponding to 50, 80, 100, 130 and 150% of the target concentration and spiked with a constant concentration of propylparaben. The regression equation was: $y = 0.0362\ (\pm 0.0003) + 0.0328\ (\pm 0.017)x$. One can notice the linear relationship ($r = 0.9999$) going through the origin, which allows to use only one standard solution for routine analysis.

3.2.5. Range

The range of the method has been set at 50–150% TMS (label claim 32.5 mg ml^{-1}) since the method was shown to be precise, accurate and linear within this region.

3.2.6. Sample solution stability

Stability of sample solution was determined after storage in the dark at room temperature. Sample preparations were analysed at 0 and 144 h. The data were evaluated as to the % content change with respect to time zero. Since the % changes are within $\pm 2\%$ sample solutions are considered stable at room temperature for up to 144 h.

3.2.7. Robustness

The influence of the different chromatografic parameters upon the separation was evaluated by systematically varying the following factors: pH of mobile phase, column temperature, SOS-concentration and percentage acetonitrile (v/v) in the mobile phase, type of column. Only one factor was changed while the others were kept constant. This study was asssessed by injecting two different solutions each time a factor was changed: one solution containing TMS, promethazine . HCl and promazine . HCl and a solution containing TMS and propylparaben.

The retention times and resolutions applying three different C18 columns are listed in Table 2. The three columns are suitable for TMS analysis. The influence of the pH of the mobile phase on the resolution between TMS and propylparaben and between TMS, prometazine . HCl and promazine . HCL, on the other hand, is illustrated in Table 3. In the studied pH-interval only little changes can be observed. The influence of the column temperature was examined at 25, 30, 35, 40 and 45°C. As expected, the resolution increased with increasing the column temperatures (Fig. 4a). SOS as an ion-pairing agent is added to the mobile phase to retain TMS, which is posi-

tively charged at pH 3.0. The SOS concentration in the mobile phase was varied in the range from 10 to 30 mM. As expected, the capacity factors decrease with decreasing SOS concentrations (Fig. 4b), although as can be seen, the resolution between TMS and propylparaben stays almost unaffected by changing the SOS-concentration. Effects of the variation of mobile phase composition were studied by applying four different mobile phases containing 25, 30, 35 or 40% (v/v) acetonitrile. By decreasing the concentration of acetonitrile, analysis times increase resulting in lower resolutions (Fig. 4c).

3.3. Application of the developed and validated method to the quantitative determination of TMS in ampoules

The validated method was succesfully applied to the quantitative determination of TMS in intramuscular injection solutions having a TMS content of 65 mg per 2 ml ampoule. The TMS content in the injection solution was found to be equal to a mean value of 100.3% (RSD 0.4%) of the label claim, analysis occurring right after batch production.

Stability studies for the mentioned TMS ampoules were also performed in the present laboratory by this stability indicating HPLC method under storage conditions of room temperature and 40°C over a period of 15 months. From Table 4 it can be concluded that TMS did not show significant degradation in the studied dosage form at room temperature and 40°C during the 15 months period. Further stability studies are still in progress.

4. Conclusions

The described isocratic reversed phase HPLC method for the determination of thiazinamium methylsulphate has been evaluated for linearity, precision, accuracy and specificity. The TMS peak response was shown to be precise, accurate and linear over the range of 50–150% (label claim: 32.5 mg ml^{-1}). Sample solutions are stable up to 144 h. Consequently the validated method for the determination of TMS may be regarded as a stability-indicating assay.

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