# DETERMINATION OF ARYLSULPHONYLBENZAMIDES RELATED PRODUCTS OF ARYLSULPHONYLTHIO-BENZAMIDES BY DERIVATIVE SPECTROMETRY

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**abstract:** A derivative spectrometric method has been developed for the determination of arylsulphonylbenzamides related products of arylsulphonylthiobenzamides. At the wavelengths selected the calibration graphs between the amplitude of the signals and the concentration of each compound were linear. The method is simple and rapid and does not require any preliminary treatment of the sample. The method was validated.

## Introduction

Derivatives of arylsulphonylthiobenyamides have unique properties connected with the simultaneous presence of sulphonyl and thiobenzamide moieties in their molecules. Such compounds have been tested and shown effective activity against *Mycobacterium tuberculosis*, variety H27 and more recently in cancer treatment [1,2,3]. Arylsulphonylbenyamides are used as raw material in the synthesis of arylsulphonylthiobenzamides [4] and can be considered as major impurities of them (Fig. 1). For the purity control of arylsulphonylthiobenzamides thin layer chromatography [4] has been reported. This paper reports a rapid, sensitive and highly selective UV derivative spectrometric method used for the simultaneously determination of arylsulphonylbenzamides and arylsulphonylthiobenzamides.
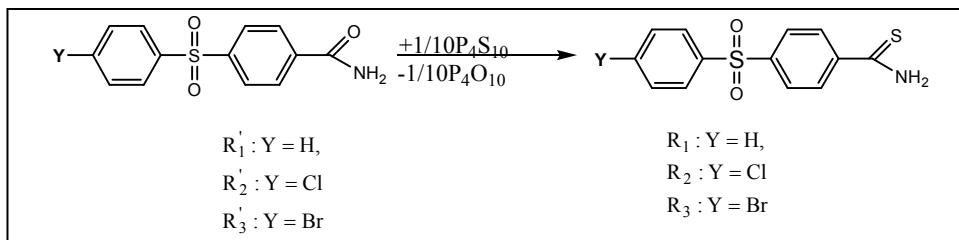


**Fig. 1.** *Synthesis of arylsulphonylthiobenzamides*

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## Experimental

### <u>Reagents</u>

All chemicals and solvents used were commercially available and were of analytical grade (obtained from Merck). The water used was double distilled. Stock solutions of each organic reagent ($10^{-3}$ mol.L$^{-1}$) were prepared by dissolving the appropriate quantity of each in methanol 99.9%. Working solutions ($5 \cdot 10^{-4}$ mol.L$^{-1}$ in methanol) were prepared by dilution of the stock solutions. A series of working solutions containing a fixed quantity of arylsulphonylthiobezamide and various quantities of corresponding arylsulphonylbezamide were prepared.

### <u>Apparatus</u>

All measurements were performed on a Jasco V 530 spectrometer coupled with a Hewlett Packard PC computer, running the Jasco software. The measurements have been made in quartz cells of 1 cm path length and conditions for recording the spectra were: wavelength range, 200-400 nm; scan speed, 100 nm/min; slit width, 2 nm; wavelength interval 1nm; smooth, 5. The ORIGIN program (Microcal Inc, version 6.0) was employed for the linear regression analysis. For ruggedness study the SPECORD M42 was used as a second spectrometer.

### <u>Procedure</u>

The UV spectra of the working solutions are recorded in the range 200-400 nm. First, second and third derivatives spectra of the zero order spectra were obtained by means of Jasco software. The zero-crossing method has been used to select the optimum wavelength for quantitative determination of arylsulphonylbenzamide in the presence of arylsulphonylthiobezamide.

*Linearity and range.* For the calibration graph successive dilutions of working solutions were performed using 25 mL volumetric flasks. The zero order spectrum of each solution was recorded against methanol as blank. The first, second and third derivative spectrum was obtained using the software supplied by Jasco.

*Precision.* Six spectra of different concentration of each compound were recorded on the same day and the values of RSD were calculated to determine the intra-day precision. The same procedure was also performed on different days and the inter-days precision was determined.

*Accuracy.* Accuracy was evaluated by fortifying a mixture containing the arylsulphonylthiobezamide with known concentrations of the arylsulphonylbenzamide. The recovery of each compound was calculated.

*Detection and quantification limits.* According to ICH [5] rules the LOD was determined based on the standard deviation of the response and the slope. The quantification limit was also calculated.

*Ruggedness.* The ruggedness was established through the spectrometric studies by different analysts on the same apparatus. A study was also performed on a different spectrometer on a different day.

## Results and discussion

Arylsulphonylthiobezamide and the corresponding arylsulphonylbenzamide are quasi similar as structure. This fact implies a very similar zero order spectra for each pair amide-thioamide studied. As it is shown in Fig. 2 the zero spectra of thioamides are not different from that of the corresponding amides. Therefore it is not possible to develop a quantitative method for the determination of the amide in the presence of thioamides based on the zero order UV spectra. The problem was solved using the derivative spectrometry.
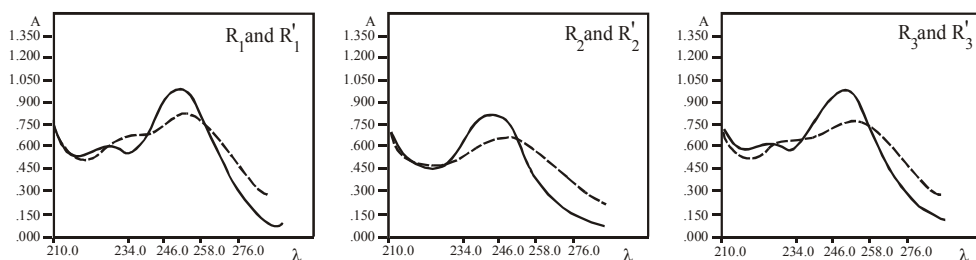


**Fig. 2.** *Zero order spectra for (——) arylsulphonylbenzamide and ( ------) arylsulphonylthiobezamide*

## Spectrometric measurements

The first derivatives spectra of $R_1$ and $R_1'$ are presented in Fig. 3. As it shown the 248 nm was selected as the optimum working wavelength for determination of $R_1'$ in the presence of $R_1$. It is possible to determine $R_1$ in the presence of $R_1'$ also. The working wavelength is 244 nm. For the pair $R_2$ and $R_2'$ the first derivatives spectra are presented in Fig. 3b. The zero-crossing point for $R_2$ is 253 nm and for $R_2'$ is 249 nm respectively. The pair $R_3$ and $R_3'$ show a better separation of bands only in the third derivatives spectra. As it is shown in Fig. 3c the zero-crossing point for $R_3$ is 230 nm and for $R_3'$ at 237 nm.
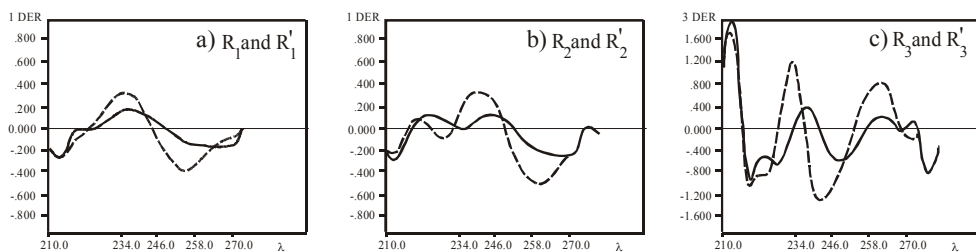


**Fig. 3.** *derivatives spectra for (——) arylsulphonylbenzamide and ( ------) arylsulphonylthiobezamide*

## Validation of the method

*Linearity and range.* The results of the linearity and range for each pair amide-thioamide are presented in Table 1. The intercept is very small and the correlation coefficient almost equal to unity. The values obtained show a good linearity and the fit of Beer's law. The

Student's t distribution was calculated. These values do not exceed the tabulated data of 2.77 for a probability of 95% means that the intercept of regression line is not significantly different from zero hence the method is free from the procedural errors. Detection limits and the quantification limits are presented in Table 1 also.

**Table 1.** Study of linearity for arylsulphonylthiobeyamide and arylsulphonylbeyamide

| Reagent | Range ($\mu g.mL^{-1}$) | $y = a + bx$ | | | LOD ($\mu g.mL^{-1}$) | LOQ ($\mu g.mL^{-1}$) |
|---|---|---|---|---|---|---|
| | | a | b | R | | |
| $R_1$ | 2.0 – 25.0 | $-35.44 \cdot 10^{-3}$ | $8.52 \cdot 10^{-2}$ | 0.9999 | 0.441 | 1.543 |
| $R'_1$ | 0.5 – 2.5 | $7.48 \cdot 10^{-3}$ | $-29.78 \cdot 10^{-3}$ | 0.9942 | 0.052 | 0.182 |
| $R_2$ | 2.0 – 25.0 | $-0.03978$ | 0.0079 | 0.9948 | 0.517 | 1.801 |
| $R'_2$ | 0.5 – 2.5 | $-0.00267$ | $-0.0160$ | 0.9995 | 0.031 | 0.108 |
| $R_3$ | 2.0 – 26.0 | 0.00048 | $-0.0309$ | 0.9945 | 0.582 | 2.037 |
| $R'_3$ | 0.5 – 2.7 | 0.02942 | $-0.0092$ | 0.9987 | 0.028 | 0.098 |

Note: R = correlation coefficient.

*Precision.* The RSD values for intra-day precision and for the inter-day precision are both bellow 2% for each pair amide-thioamide. These results confirm that the method is precise.

*Accuracy.* In order to verify the accuracy six replicate determination were performed on each of five solid mixture containing arylsulphonylthiobenzamide (20 $\mu g \cdot mL^{-1}$) and arylsulphonylbenzamide (0.5 $\mu g \cdot mL^{-1}$). Percentage recovery was calculated and the values obtained are in the range 98 – 102%.

**Table 2.** Determination of arylsulphonylthiobeyamide and arylsulphonylbeyamide in synthetic samples

| Reagent | sample no. | Quantity, mg | | | | |
|---|---|---|---|---|---|---|
| | | $R_i$ | | $R'_i$ | | |
| | | actual (%) | determined (%) ± s.d. | actual (%) | determined (%) ± s.d. | |
| $R_1$ and $R_1'$ | 1 | 100.0 | 98.62 ± 0.14 | 2.0 | 98.12 ± 0.02 | |
| | 2 | 100.0 | 101.3 ± 0.54 | 5.0 | 102.3 ± 0.02 | |
| | 3 | 100.0 | 101.2 ± 0.71 | 10.0 | 99.24 ± 0.03 | |
| $R_2$ and $R_2'$ | 1 | 100.0 | 100.3 ± 0.65 | 2.0 | 101.4 ± 0.02 | |
| | 2 | 100.0 | 100.1 ± 1.05 | 5.0 | 100.8 ± 0.02 | |
| | 3 | 100.0 | 100.1 ± 0.98 | 10.0 | 98.53 ± 0.03 | |
| $R_3$ and $R_3'$ | 1 | 100.0 | 100.3 ± 0.71 | 2.0 | 99.47 ± 0.02 | |
| | 2 | 100.0 | 100.12 ± 0..28 | 5.0 | 100.5 ± 0.02 | |
| | 3 | 100.0 | 99.97 ± 0.64 | 10.0 | 99.52 ± 0.03 | |

Note: i = 1,3; each value represents the average of three determinations ± standard deviation.

*Stability of the solutions.* The stock solutions of the each compound were stored in light at room temperature for 30 days. The solutions were analyzed twice a week during the whole period. The results obtained by the proposed method were compared with those obtained using TLC method. No degradation products were observed during this time.

*Ruggedness.* Ruggedness was performed to confirm that the assay of each organic reagent was satisfactory under conditions external to the method. Good results were obtained during this study confirming that the method remained selective and precise for all components under tested conditions.

*Applications.* Synthetic samples containing each pair arylsulphonylthiobeyamide and arylsulphonylbeyamide amide have been analyzed by two different methods. The results are presented in Table 2 and show good recovery.

## Conclusion

A new derivative spectrometric method was proposed for simultaneous determination of arylsulphonylbenzamide and arylsulphonylthiobenzamide in synthetic mixture. The method was validated and can be used not only for the determination of thiobezamide and the corresponding amide but also for the routine control of the synthesis of the thiobenyamide. The method is simple, selective and rapid.

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