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Short Communication

Problems of semiquantitative TLC methods prescribed in the United States and British Pharmacopoeias for the purity testing of sulphinpyrazone

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Introduction

Sulphinpyrazone is official in the USP XXI and BP1980 pharmacopoeias. To determine its purity thin-layer chromatographic (TLC) methods are used [1, 2]. Although the stationary phase and eluent are either identical or very similar, the many slight changes in the experimental conditions produce different results for the same sample. Hence the pretreatment of the sorbent, the inert gas used for protection of sulphinpyrazone and the evaluation and quantification of the chromatograms differ in the respective pharmacopoeial methods.

The main source of error in the test in the experience of the authors is the possible decomposition of sulphinpyrazone during the analysis which is partly dependent on the skill and expertise of the analyst. Another problem is the quantification of the impurities: estimation by visual comparison of the spots with those of reference materials [1] or to the spot of sulphinpyrazone RS [2]. In the latter case the evaluation is more difficult, because of the different R_f-values of impurity spots and sulphinpyrazone, and also because of the decomposition of sulphinpyrazone RS on the layer, resulting in apparently higher impurity levels when the impurities are expressed as sulphinpyrazone. It should be noted that owing to the decomposition of reference material during the test in the USP system, the lowest applied quantity of sulphinpyrazone (0.2 μg, equivalent to 0.2% of impurity) cannot be detected by visual observation.

The main aim of the present work was to investigate the problems associated with the BP and USP TLC methods, to find chromatographic conditions such that the degradation is minimized and to increase the accuracy and precision of the evaluation of the chromatoplates by use of spectrodensitometry.

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The following parameters have been investigated:
(i) The effect of the original BP and USP systems on the decomposition of sulphinpyrazone;
(ii) The effect of a modified eluent system enabling densitometric evaluation of the separated zones;
(iii) The use of concentrating zone silica gel plates; and their effect on the decomposition of sulphinpyrazone;
(iv) The effect of the antioxidant, used in the eluent and sample solution, on the decomposition;
(v) Comparison of the separations obtained by the optimized TLC method and a HPLC method.

Experimental

Materials

2,6-Di-tert-butyl-4-methylphenol (BHT) was USP quality, the solvents used for HPLC were of HPLC-grade quality, the other solvents and reagents were of analytical grade and were obtained from Reanal, Budapest, Hungary. The reference materials were of BPCRS quality.

Equipment

A Varian 8500 high-performance liquid chromatograph equipped with variable wavelength UV-detector, loop injector and electronic integrator (Varian AG, Walnut Creek, CA, USA) was used for the HPLC experiments.

An Opton KM-3 chromatogram spectrophotometer (Opton Feintechnik GmbH, Oberkochen, FRG) was used for the spectrodensitometric evaluation.

Chromatographic conditions

Thin-layer chromatography. The separations were achieved using precoated silica gel 60 F_{254} (Part No. 5715) and silica gel 60 F_{254} with concentrating zone (Part No. 11798) chromatoplates (E. Merck, Darmstadt, FRG). The pre-treatment of the layers was carried out as directed in the Pharmacopoeias [1, 2]. The mobile phase was chloroform–glacial acetic acid (80:20, v/v or 95:5, v/v).

The sample and reference solutions were spotted 1 cm apart on the chromatoplate.

After development the chromatograms were evaluated visually as prescribed in the Pharmacopoeias and by spectrodensitometric measurements at 265 nm.

*High performance liquid chromatography.** Column: prepacked 10 μm Nucleosil C_{18} (250 × 4.6 mm i.d.) supplied by Chrompack B.V., Middleburg, The Netherlands. Eluent: acetonitrile–water (60:40, v/v) adjusted to pH 2.50 by addition of phosphoric acid with a flow rate of 1.0 ml min^{-1}. Detection: by UV measurements at 265 nm.

Results and Discussion

Comparison of the original BP and USP methods

The densitograms obtained for a sulphinpyrazone sample examined using the prescribed Pharmacopoeial procedures [1, 2] are shown in Fig. 1.

*This is a slightly modified version of the system developed at Novopharm Ltd (Scarborough, Canada).

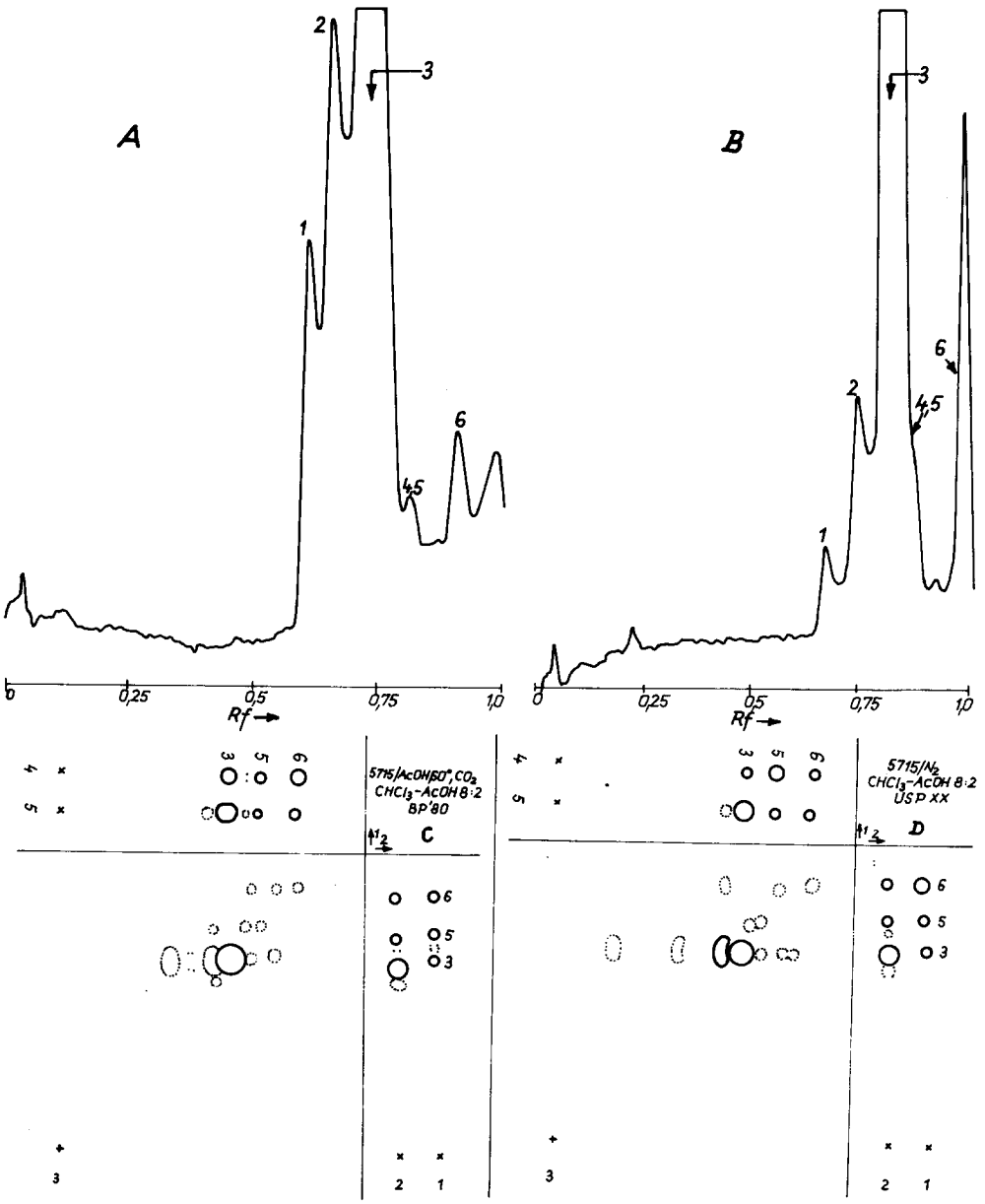

Figure 1
Densitograms of sulphinpyrazone using the original BP and USP systems. (A) BP system; (B) USP system. Conditions: Kieselgel 60 F_{254} plate, eluent: chloroform–acetic acid (80:20, v/v) evaluation at 265 nm in reflectance mode. Other conditions: same as prescribed in BP1980 [1] and USP XXI [2]. Compounds: **1**, **2** and **4**, unidentified decomposition products; **3**: sulphinpyrazone; **5**, 1,2-diphenyl-4-(2-phenylsulphonylethyl) pyrazolidine-3, 5-dione; **6**, 1,2-diphenyl-4-(2-phenylthioethyl)pyrazolidine-3,5-dione.

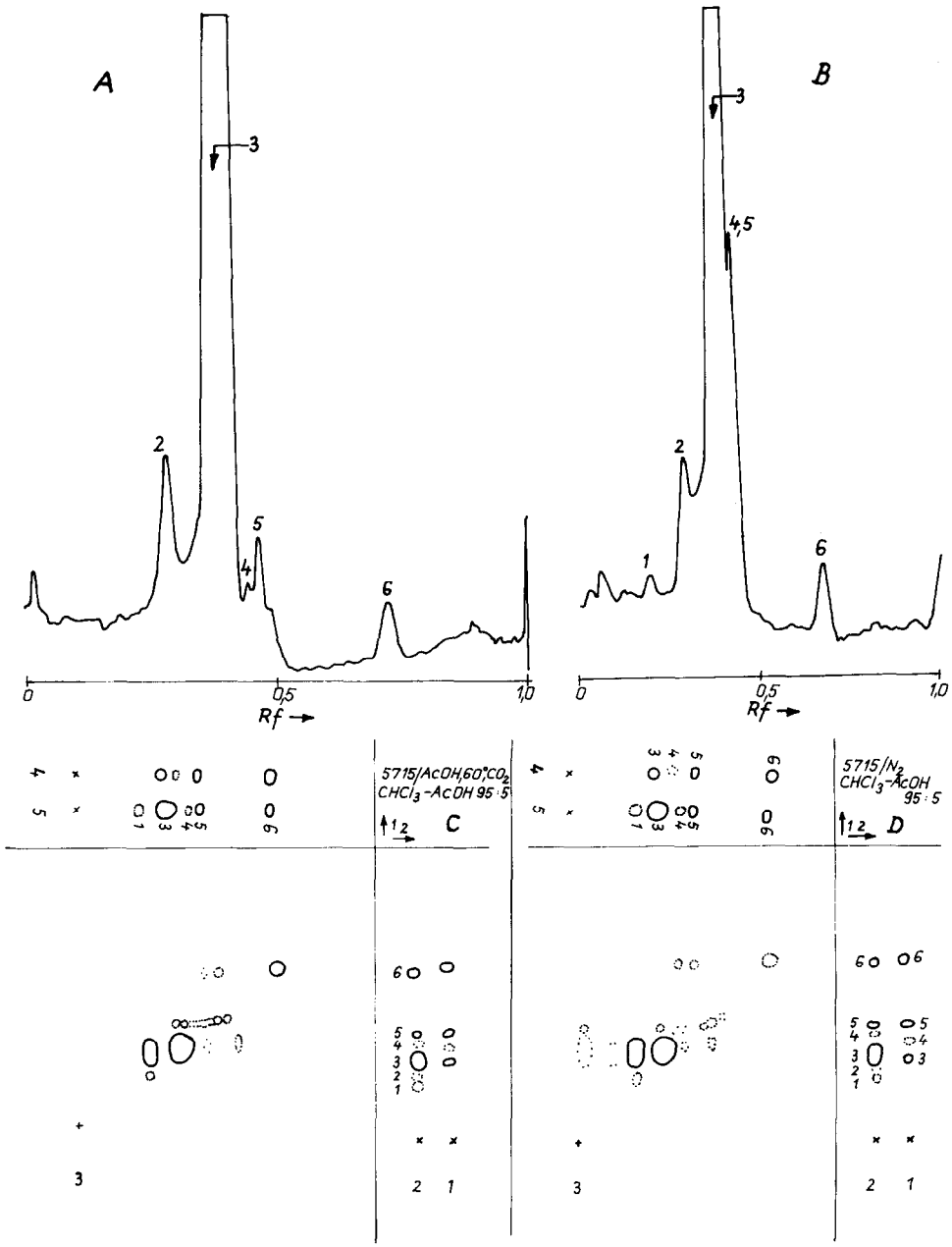

Figure 2
Densitograms using the modified BP and USP systems. (A) BP system; (B) USP system. Eluent: chloroform–acetic acid (95:5; v/v). Other conditions and compounds as in Fig. 1.

As can be seen, the evaluation of the chromatograms by densitometry is difficult because of the poor resolution of some of the components and their proximity to the solvent front. Furthermore, there is evidence that decomposition products are partly formed during chromatography. This was confirmed by two-dimensional chromatography whereupon significant evidence of analyte decomposition was apparent in the second direction of eluent flow.

Modification of eluent system

The densitograms obtained for the same sulphinpyrazone sample in the modified chloroform–acetic acid eluent system, which was found to give the optimum resolution of the various components, is shown in Fig. 2. The other experimental conditions were as directed in BP1980 (Fig. 2A) and USP XXI (Fig. 2B).

As it can be seen from Fig. 2, better resolution for the compounds investigated can be achieved, thus providing better conditions for densitometric evaluation.

The above figures also demonstrate that the use of a carbon dioxide or nitrogen atmosphere during sample introduction and development cannot prevent decomposition completely. However, probably due to higher density of the carbon dioxide used, the BP system results in less decomposition.

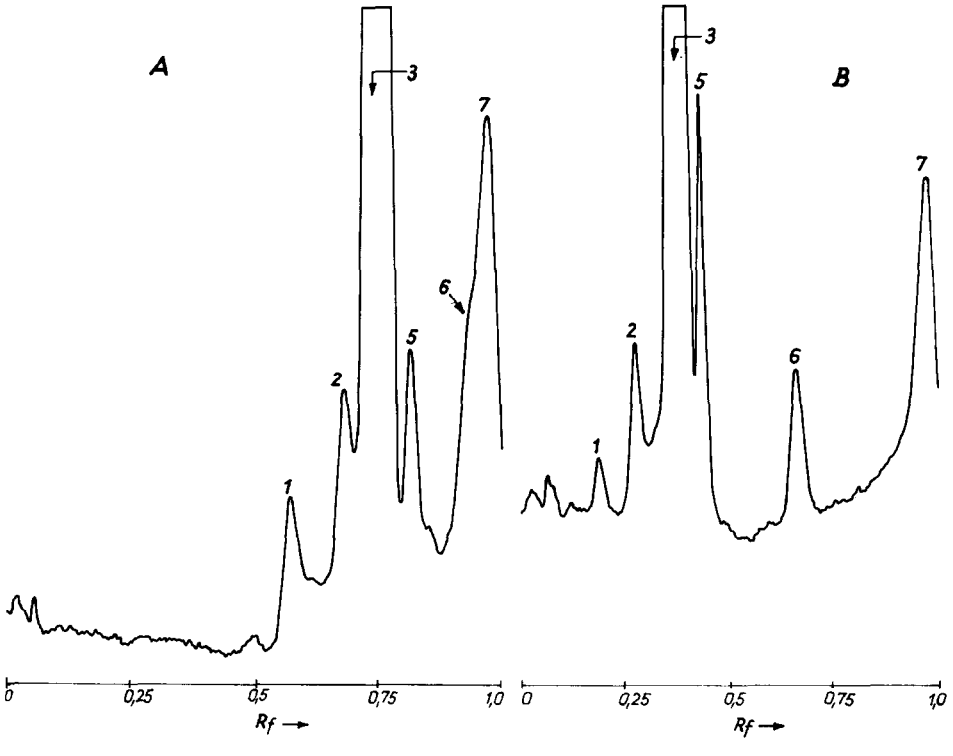

Figure 3
Densitograms of sulphinpyrazone in the BP systems using BHT in the eluent and sample solution. (A) Original BP system; (B) modified BP system. Eluent contains 0.02% BHT; sample solution contains 0.1% BHT. Other conditions: same as in Fig. 1. Compounds: 7, 2,6-di-tert-butyl-4-methylphenol; other compounds as in Fig. 1.

Decomposition during chromatography

In order to minimize decomposition due to oxidation the antioxidant 2,6-di-tert-butyl-4-methylphenol (BHT) was added to both the eluent and the sample solution.

The experimental results reveal that a significant decrease in decomposition can be achieved by using BHT both in the eluent and sample solution. The optimal concentration of BHT was found to be 0.02 m/v % in the eluent and 0.1 m/v % in the sample solution.

The densitograms of sulphinpyrazone sample, using the original (Fig. 3A) and modified BP (Fig. 3B) systems containing BHT in both the eluent and sample solution at an optimum concentration, are shown in Fig. 3. Similar results were obtained using the USP system.

Decomposition during sample application

In order to minimize the decomposition occurring during sample application and drying of the spot prior to development, silica gel TLC plates with concentrating zones have also been used.

Figure 4 shows the densitograms obtained in the original (Fig. 4A) and modified BP systems (Fig. 4B). The other conditions were the same as shown in Fig. 3.

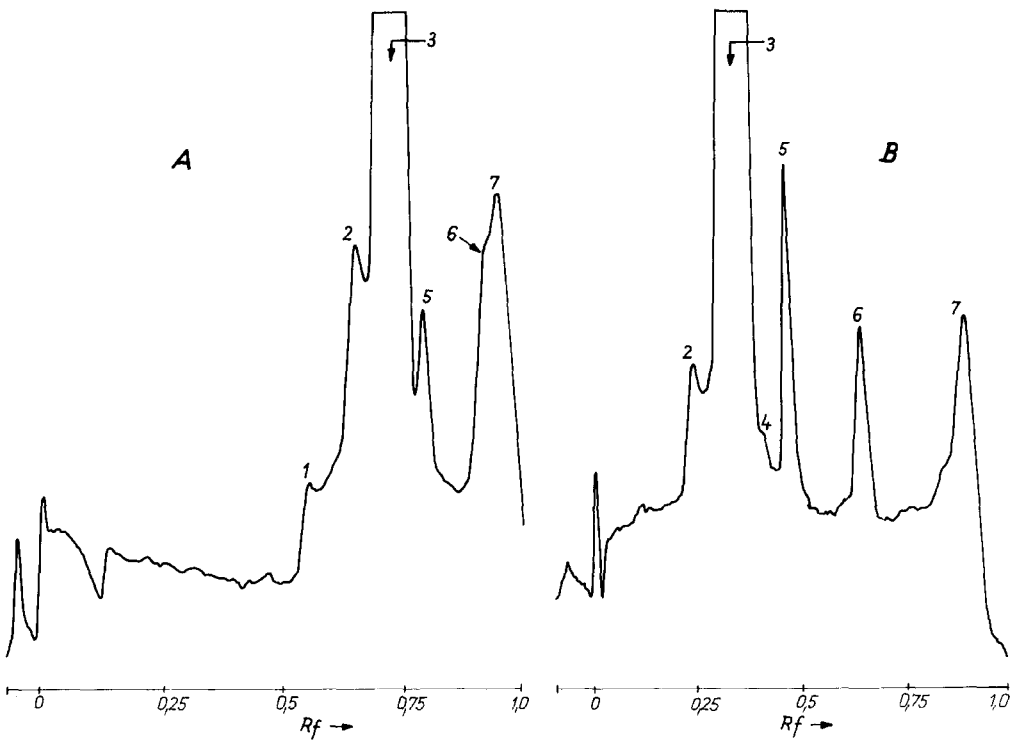

Figure 4
Densitograms of sulphinpyrazone in BP systems using silica gel with concentrating zone plate and BHT. (A) Original BP system (B) modified BP system. Plate: silica gel with concentrating zone. Other conditions and compounds as in Fig. 3.

It should be appreciated that when BHT is used in the sample solution and in the eluent, an additional spot due to BHT is observed, however, its presence does not affect the determination.

The experimental results clearly demonstrate that the use of silica gel plates with a concentrating zone and the addition of BHT to the eluent and sample solution provides the best conditions for the correct measurement of impurity level in sulphinpyrazone by quantitative TLC.

Application of the modified BP system leads to no additional decomposition as confirmed by two-dimensional chromatography.

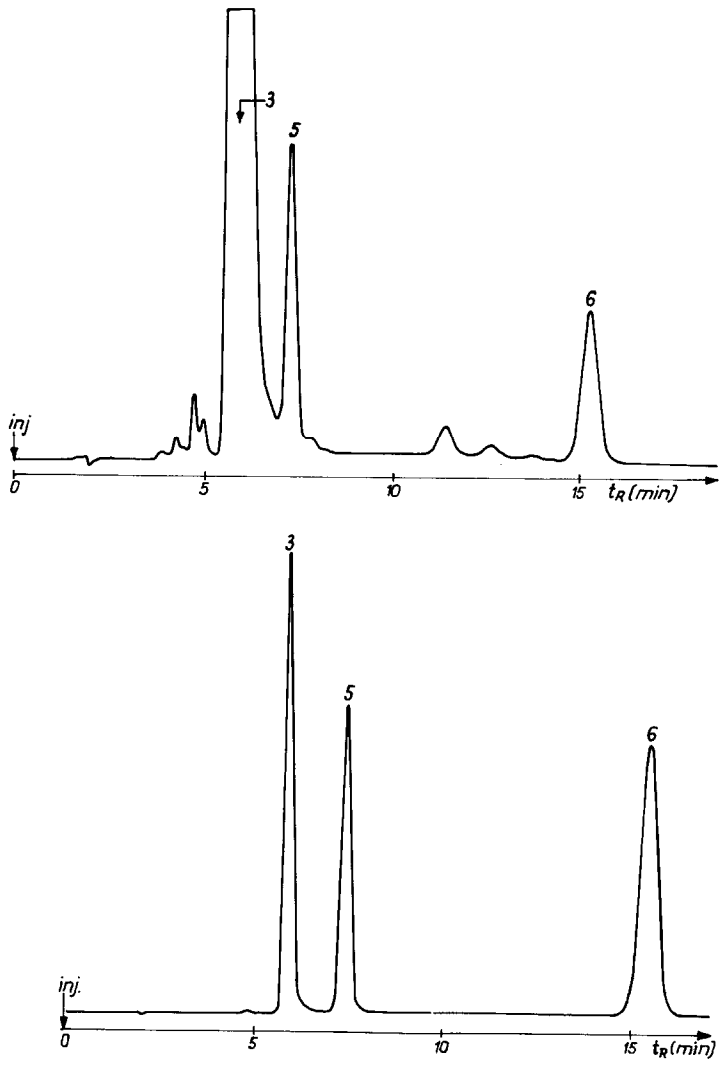

Figure 5
Separation of sulphinpyrazone and its impurities by HPLC. (A) Chromatogram of a standard mixture; (B) chromatogram of a sulphinpyrazone sample. Conditions: column; 10 μm Nucleosil C_{18} (250 × 4.6 mm i.d.); eluent; acetonitrile–water (60:40, v/v) adjusted to pH 2.5 with phosphoric acid; flow rate; 1 ml min^{-1}, detection at 265 nm, compounds as in Fig. 1.

Comparison of the TLC and HPLC separations

The chromatogram obtained for the same sulphinpyrazone sample by HPLC is shown in Fig. 5.

As shown in Fig. 5 an excellent resolution of sulphinpyrazone and its impurities can be obtained by HPLC. The HPLC system is characterized by a resolution between sulphinpyrazone and 1,2-diphenyl-4-{2-phenylsulphonylethyl}-pyrazolidine-3,5-dione of not less than 1.4, an asymmetry factor measured for sulphinpyrazone of less than 1.5 and a reproducibility of better than 2% for the main component and 5% for the impurities present in the sample at approximately the 1% level.

The main advantage of the HPLC method is suppression of the analyte decomposition. Normally the same impurity level is measured after leaving the sample solution standing for 60 min at room temperature. The method has been routinely used in the author's laboratory for about 6 months.

Conclusion

As the result of a comparison of the TLC methods described in the USP XXI and BP1980 pharmacopoeias the following conclusions can be drawn. Both systems have some problems resulting from the decomposition of sulphinpyrazone and related substances during the test. Furthermore, the semiquantitative estimation of the impurities owing to the high R_f-values is questionable. However, by adoption of the proposed changes, namely that separation is performed on concentrating zone silica gel plates with BHT in the sample solution and the modified eluent system, applying spectrodensitometric evaluation of the separated spots together with the use of the plate pre-treatment procedure and use of an inert gas as described in BP1980, the main sources of error can be eliminated. The HPLC method described can be used more advantageously for the purity testing of sulphinpyrazone considering the better chromatographic resolution and higher accuracy and precision of the method. For this reason its use can be recommended as a pharmacopoeial method.

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References

[1] *British Pharmacopoeia*, Vol. I, p. 439. Addendum 83, p. 255. HMSO, London (1980).
[2] *United States Pharmacopoeia* XXI, p. 998. USP Convention Inc., Rockville, MD. (1984).

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